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This is a request for filing a

- ☒ new utility patent application under 37 CFR 1.53(b)(1).  
☐ continuation-in-part under CFR 1.53(b)(2) of prior  
application serial no. \_\_\_\_\_, filed \_\_\_\_\_ (list  
entire parentage).

Title: NOVEL TRAF FAMILY PROTEINS

Inventor(s) (full name of each inventor): JUAN M. ZAPATA  
JOHN C. REED

Enclosed are:

- ☒ Return receipt postcard  
☒ Patent Application Bibliographic Data Sheet  
☒ 1 Page application cover sheet  
☒ 90 Pages of specification (includes claims and abstract)  
☒ 13 Sheets of drawing(s)  
\_\_\_\_\_ Pages of an executed Declaration for Patent Application  
\_\_\_\_\_ An executed Power of Attorney for Patent Application by  
\_\_\_\_\_ Assignee  
☒ Paper copy of sequence listing, pages 1 through 55  
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☒ Statement Under 37 CFR 1.821(f)  
\_\_\_\_\_ An executed assignment and cover sheet  
\_\_\_\_\_ An executed Statement Under 37 CFR 3.73(b)  
\_\_\_\_\_ An executed small entity statement  
\_\_\_\_\_ Also enclosed: \_\_\_\_\_

\_\_\_\_\_ This application is based on prior foreign application(s) No.(s) \_\_\_\_\_  
\_\_\_\_\_, filed in \_\_\_\_\_ on \_\_\_\_\_,  
respectively, and priority is hereby claimed therefrom.

\_\_\_\_\_ This application is based on, and claims the benefit of, U.S.  
Provisional Application No. 60/\_\_\_\_\_, filed \_\_\_\_\_, and  
entitled \_\_\_\_\_, and which is incorporated herein by  
reference.

☒ This application is based on, and claims the benefit of, U.S.  
Provisional Application No. 60/\_\_\_\_\_ (yet to be assigned), filed  
November 5, 1999, which was converted from U.S. Serial No.  
09/434,784, and entitled NOVEL TRAF FAMILY PROTEINS, and which is  
incorporated herein by reference.

Inventors: Zapata and Reed  
Docket No.: P-LJ 4453  
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The filing fee has been calculated as shown below:


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## APPLICATION INFORMATION

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UNITED STATES LETTERS PATENT

on

NOVEL TRAF FAMILY PROTEINS

by

Juan M. Zapata

and

John C. Reed

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**NOVEL TRAF FAMILY PROTEINS****BACKGROUND OF THE INVENTION**

This application claims the benefit of U.S. Provisional Application No. 60/\_\_\_\_, filed  
5 November 5, 1999, which was converted from U.S. Serial No. 09/434,784, filed November 5, 1999, and is incorporated herein by reference.

**FIELD OF THE INVENTION**

10 This invention relates generally to the fields of molecular biology and molecular medicine and more specifically to proteins involved in the regulation of immunological response and cell death.

15 This invention was made with government support under grant number CA69381 awarded by the National Institute of Health. The United States Government has certain rights in this invention.

Tumor Necrosis Factor (TNF) family cytokines play an important role in a wide variety of immunological, allergic, and inflammatory responses.  
20 Several members of the TNF family have been identified, including TNF $\alpha$ , Lymphotoxin- $\alpha$ , Lymphotoxin- $\beta$ , LIGHT, CD27 Ligand (CD27L), CD30L, CD40L, Fas-L, Trail, and others. These molecules are generally produced as Type-II integral membrane proteins on the surface of cells,  
25 undergoing subsequent release into the extracellular milieu as a result of proteolytic cleavage. Many of the TNF-family cytokines however remain anchored in the plasma membrane, relying on interactions with receptor-bearing cells through cell-cell contact.

The receptors for TNF-family cytokines are equally diverse. All members of the family have a conserved arrangement of cysteines in their extracellular domains, which is one of the criteria for membership in this family. The intracellular cytosolic domain of TNF-family receptors are diverse in their amino acid sequences, but can be broadly classified into two types: (a) those that contain a protein-interaction module known as a Death-Domain (TNFR1, Fas, DR3, DR4, DR5) and those that do not (TNFR2, CD27, CD30, CD40, LT $\beta$ R, 4B1 and others).

Death Domains are responsible for interactions of a subgroup of the TNF-Receptor (TNFR) family with adapter proteins which bind in turn to caspase-family intracellular proteases involved in inducing apoptosis (programmed cell death). However, the Death Domains can also mediate binding to other types of adaptor molecules which bind kinases or other types of signaling molecules rather than proteases.

Those TNFR family members that do not contain a Death Domain in their cytosolic tail rely on a family of intracellular adapter proteins for transducing signals. This family of adaptor proteins is known as the "TNF Receptor Associated Factors" (TRAFs). TRAF-family proteins contain a protein interaction domain known as the TRAF-domain that mediates their binding to the cytosolic domains of TNF-family receptors. The TRAF domain also allows for interactions among TRAF-family members, creating opportunities for homo- and hetero-oligomerization that can have important functional consequences. In humans and mice, six members of the TRAF family have been described, including TRAF1, TRAF2, TRAF3, TRAF4, TRAF5, and TRAF6. The crystal structure of

the TRAF domain of TRAF2 has been solved, revealing a trimeric assembly with 3-fold symmetry and demonstrating the presence of a surface pocket on each monomer that accounts for binding to discrete peptidyl motifs found within the cytosolic domains of many members of the TNF-family receptors.

Some TRAF family proteins physically associate with protein kinases and control the activation of these kinases. TRAF2, TRAF5, and TRAF6 for example have been reported to be capable of inducing the activation of kinases involved in activation of: (a) several members of the stress-kinase family, such as the Jun N-terminal kinase (JNK), and (b) phosphorylation of I $\kappa$ B, an inhibitor of the transcription factor NF- $\kappa$ B. In contrast, TRAF1, TRAF3, and TRAF4 do not activate these kinase, and in some contexts, may interfere with kinase activation by other TRAFs. All kinase-activating members of the TRAF family contain additional protein domains (besides the TRAF-domain) which are important for their function as kinase-activators, including a RING domain and sometimes zinc-finger domains.

Gene knock-out studies in mice have demonstrated critical roles for several of the TRAF-family proteins in signal transduction pathways stimulated by TNF-family receptors. Moreover, mutational analyses of the TRAF-binding sites within the cytosolic domains of TNF-family receptors have also provided evidence that interactions of TRAFs with these receptors are critical for many of the biochemical signal-transduction and cellular biological responses induced via TNF-family receptors.



In addition to their involvement in signaling by TNF-family receptors, at least one of the known TRAFs can also participate in signaling mediated by the Interleukin-1 Receptor/Toll family of receptors. TRAF6  
 5 interacts with a Death Domain-containing adapter protein (MyD88) and a Death Domain-containing protein kinase (IRAK) which are critical for NF- $\kappa$ B induction by IL-1 receptors. Thus, TRAFs may in some instances participate in signal transduction by other cytokine receptors beside  
 10 those of the TNFR family. In addition, some TRAFs bind to viral proteins, suggesting that such protein interactions may play a role the mechanisms employed by viruses to either evade immune surveillance mechanisms or in virus-mediated malignant transformation.

15 A need exists, therefore, to identify novel TRAF family proteins or TRAF protein binding domains. The present invention satisfies this need and provides additional advantages as well.

#### **SUMMARY OF THE INVENTION**

20 In accordance with the present invention, there are provided novel TRAF-Protein-Binding-Domain polypeptides (TPBDs). The invention also provides nucleic acid molecules encoding TPBDs, vectors containing these nucleic acid molecules and host cells containing  
 25 the vectors. The invention also provides antibodies that can specifically bind to invention TPBDs. Such TPBDs and/or anti-TPBD antibodies are useful for discovery of drugs that suppress autoimmunity, inflammation, allergy, allograft rejection, sepsis, and other diseases.

30 The present invention also provides a screening assay useful for identifying agents that can effectively

alter the association of an invention TPBD with itself or with other proteins. By altering the self-association of TPBD or by altering their interactions with other proteins, an effective agent may increase or decrease the activation of kinases, or modulate cellular pathways that effect apoptosis, cell proliferation, cell adhesion, cell stress responses, B cell immunoglobulin class switching, and the like.

The invention also provides methods of altering the activity of TPBD in a cell, wherein such increased or decreased activity of TPBD can modulate the level of kinase activity or cellular pathways that effect apoptosis, cell proliferation, cell adhesion, cell stress responses, B cell immunoglobulin class switching, and the like. For example, the activity of TPBD in a cell can be increased by introducing into the cell and expressing a nucleic acid sequence encoding this polypeptide or proteins comprising such TPBD. In addition, the activity of TPBD or TPBD-comprising proteins in a cell can be decreased by introducing into the cell and expressing an antisense nucleotide sequence that is complementary to a portion of a nucleic acid molecule encoding the TPBD or TPBD-comprising proteins.

The invention also provides methods for using an agent that can specifically bind TPBD or a nucleotide sequence that can bind to a nucleic acid molecule encoding TPBD to diagnose a pathology that is characterized by an altered level of apoptosis, cell proliferation, cell adhesion, cell stress responses and B cell immunoglobulin class switching due to an increased or decreased level of TPBD in a cell.

# **BRIEF DESCRIPTION OF THE FIGURES**

Figure 1 shows a schematic representation of the structure of newly identified TRAF proteins referred to as HAUSP (SEQ ID NO:8) (also known as USP7), SPOP (SEQ ID NO:10) and TRAF 7 (SEQ ID NO:12); and additionally, TRAF 1 and TRAF 2, showing in shaded boxes the relative positions of the indicated protein domains: TRAF domain, protease domain, ring finger, Z-b box, zinc finger, poly-acidic domain, coiled coil, and nuclear localization signal.

Figure 2 shows amino acid sequences of the TRAF domains of TRAF 7 (SEQ ID NO:25) (also known as KIAA), HAUSP (SEQ ID NO:23) (also known as USP7), and SPOP (SEQ ID NO:24) in alignment with TRAF domains of six other known human TRAF proteins (hT1td through hT6td, SEQ ID NOS:26-31). Dark boxes indicate identical residues between family members. Light boxes indicate structurally related residues.

Figure 3 shows the predicted amino acid sequence of TRAF7 (SEQ ID NO:32, corresponding to amino acids 16-979 of SEQ ID NO:6). The different protein domains and regions of the protein are indicated: ring finger domain (amino acids 15-55); ZF-B Box domain (amino acids 90-132); coiled coil (amino acids 132-177); coiled coil (amino acids 195-231); two leucine zipper domains (amino acids 197-218 and 222-245); TRAF domain (amino acids 277-403); coiled coil (amino acids 427-446); and two poly-acidic regions (amino acids 868-964).

Figure 4 shows Northern blot analysis of the mRNA levels of TRAF7, USP7 and SPOP in human tissues.

Northern blot analyses were performed using the human 12 lane multiple tissue northern blot (MTN; Clontech; Palo Alto CA), as recommended by the manufacturer. The TRAF domains of TRAF7, USP7 and SPOP, as well as actin as a control, were labeled with  $^{32}\text{P}$ -cytidine using a nick translation assay kit.

Figure 5 shows an analysis of the binding of the invention TRAF-protein-binding-domains (TPBDs) to different members of the TNF receptor family. *In vitro* binding assay results of the cytosolic domains of selected members of the TNF-R family are presented. GST-Fas(ct), GST-TNF-R2(ct), GST-CD40(ct), GST-LT $\beta$ R(ct), GST-NGFR(ct) and GST-DR4(ct) immobilized on glutathione-Sepharose were incubated with *in vitro* translated ( $^{35}\text{S}$ )-TRAF2, -SPOP, -HAUSP, and -TRAF7 TRAF domains. Alternatively, TRAF domains of TRAF7 or TRAF2 were expressed in 293 cells. Bound TRAF proteins were detected by SDS-PAGE and fluorography.

Figure 6 shows an analysis of the binding of human TRAF proteins 1-6 and I-TRAF (Rothe et al., Proc. Natl. Acad. Sci. USA 93:8241-8246 (1996)) to TRAF-7, HAUSP and SPOP. GST-TRAF7 (282-435), GST HAUSP (1-213) and SPOP (1-180) proteins immobilized on glutathione-Sepharose were incubated with *in vitro* translated  $^{35}\text{S}$ -TRAF proteins and I-TRAF, as indicated. Bound TRAF proteins were detected by SDS-PAGE and fluorography.

Figure 7 shows that TPBDs of HAUSP, SPOP and TRAF7 can specifically inhibit the NF- $\kappa\text{B}$  activity induced by TRAF containing proteins. In upper panel relative NF- $\kappa\text{B}$  activities effected by different TRAF domains are shown. Cells were transfected with control plasmid,

pcDNA3-myc-hTRAF6 alone or with 7 $\mu$ g of either pcDNA3-myc-HAUSP(1-213) or pcDNA3-myc-SPOP(1-180), together with 0.5 $\mu$ g pUC13-4xNF $\kappa$ B-luc plasmid and 1  $\mu$ g pCMV- $\beta$ -galactosidase plasmid, as indicated. Relative NF- $\kappa$ B activity was assessed by luciferase assays, with normalization for  $\beta$ -galactosidase activity. The results are presented as fold of activation relative to the control.

Figure 8 shows the effect of TRAF7 and USP7 in regulating NF $\kappa$ B activity induced by TRAF2 and TRAF6 in a mammalian cell line.

Figure 9 shows the effect of the TRAF domains of TRAF7, USP7 and SPOP in regulating the NF $\kappa$ B activity induced by TNF $\alpha$  or CD40 overexpression.

Figure 10 shows regulation of NIK induced NF $\kappa$ B activation by TRAF7 and USP7.

Figure 11 shows the subcellular localization of different TRAF7 deletion mutants.

Figure 12 shows the subcellular localization of different domains of USP7.

### **DETAILED DESCRIPTION OF THE INVENTION**

In accordance with the present invention, there are provided novel TRAF-protein-binding-domains (TPBDs) of newly identified TRAF proteins, and fragments thereof. As used herein, an invention TPBD refers to a peptide region that binds to one or more TRAF proteins. Invention TPBDs share sequence homology with the C-TRAF

domain of TRAF proteins, and have been found herein to have binding properties similar to those of other known TRAF proteins. This domain corresponds to the portion of the TRAF domain commonly referred to as the C-TRAF or

5 TRAF-C domain (Arch et al., supra.; and Wajant et al., Cytokine Growth Factor Rev. 10:15-26 (1999), each of which is incorporated herein by reference). Previously characterized human TRAF domain proteins have also been found to have N-TRAF domains immediately amino-terminal

10 of the C-TRAF domain.

TRAF proteins were initially identified as a family of proteins that are associated with members of the TNF-receptors family. TRAF proteins are known to influence a variety of cellular processes such as class

15 switching in B cells, apoptosis, cell proliferation, and stress response (Arch et al., Genes Dev. 12:2821-2830 (1998)). These effects on cellular process are thought to occur by TRAF-mediated modulation of NF- $\kappa$ B and/or cJun N-terminal kinase (JNK) activity. TRAF proteins thus

20 represent an important class of intracellular proteins that mediate the signal transduction of cell surface receptors such as TNFR-family members that ultimately influence a variety of cellular processes.

In addition to binding TNF-family receptors,

25 TRAF domain proteins also interact with other TRAF domain proteins. For example, TRAF 1, TRAF 2 and TRAF 3 all have been shown to homo-oligomerize. TRAF 2 has further been demonstrated capable of hetero-oligomerization with TRAF 1 and TRAF 5. As shown herein, TRAF domains of the

30 present invention have all been demonstrated as capable of interacting with other TRAF-domain proteins.

The functions of the TRAF domain containing proteins, generally, supports the role of invention TPBDs and invention TRAF domain proteins in cellular pathways that effect apoptosis, cell proliferation, cell adhesion, 5 cell stress responses and B cell immunoglobulin class switching.

For example, invention TPBDs have been found to associate with other proteins, including proteins comprising TRAF domains. Exemplary TRAF proteins to 10 which invention TPBDs bind are human TRAF1, TRAF2, TRAF3, TRAF4, TRAF5 or TRAF6. As used herein, the term "bind" or "binding" refers to the association of an invention TPBD with another protein relatively specifically and, therefore, can form a bound complex. In particular, the 15 binding of a TPBD to a TRAF protein is sufficiently specific such that the bound complex can form *in vivo* in a cell or *in vitro* under suitable condition.

In one embodiment, it has been found that the invention HAUSP TPBD (SEQ ID NOS:8 or 23) binds TRAF1, 20 TRAF2, TRAF3, TRAF4, TRAF5 and TRAF6, and can inhibit the NF- $\kappa$ B activation activity of TRAF2, TRAF5 and TRAF6. In another embodiment, it has been found that the invention SPOP TPBD domain (SEQ ID NOS:10 or 24) binds TRAF1 and TRAF6, and can inhibit the NF- $\kappa$ B activation activity of 25 TRAF6. In yet another embodiment, it has been found that the invention TRAF-7 TPBD (SEQ ID NOS:12 or 25) binds TRAF1, TRAF2, TRAF3, TRAF4, TRAF5 and TRAF6, and can inhibit the NF- $\kappa$ B activation activity of TRAF2 and TRAF6, and can increase the NF- $\kappa$ B activation activity of TRAF5. 30 Furthermore, it has been found that the TRAF domain of TRAF7 and USP7 can inhibit the NF- $\kappa$ B activation induced

by TNF $\alpha$  and CD40 overexpression, whereas the TRAF domain of SPOP showed no inhibitory activity.

In an additional embodiment, it was found that TRAF7, the TRAF domain of TRAF7, and the TRAF domain of USP7 (HAUSP) inhibited NIK-induced NF $\kappa$ B activation. Therefore, the invention provides TPBDs that can be used to inhibit NIK-induced NF $\kappa$ B activation.

In another embodiment, invention TPBDs also bind to TNF receptor family proteins. For example, the SPOP TPBD of SEQ ID NO:10 binds TNF-R2, and the TRAF-7 TPBD (SEQ ID NOS:12 or 25) binds to TNF-R2, CD40, lymphotoxin- $\beta$ R, NGFRp75 and DR4, and the TRAF domain of TRAF7 also binds with itself. Further, TRAF proteins generally, are known to bind to a wide variety of cell-surface receptors, and in particular, with cytokine receptors of the TNF receptor family. Exemplary receptors to which TRAF proteins are known to bind are: TNFR1, TNFR2, CD27, CD30, CD40, 4-1BB, Ox40, LT- $\beta$ R, Fas, DR3, DR4, DR5, HVEM, LMP-1 and IL-1R. In addition, TRAF proteins have been observed to bind to all members of the TNF receptor family that do not comprise a death domain. Thus, it is contemplated herein that invention TPBDs also bind one or more receptors selected from TNFR1, TNFR2, CD27, CD30, CD40, 4-1BB, Ox40, LT- $\beta$ R, Fas, DR3, DR4, DR5, HVEM, LMP-1, IL-1R, and members of the TNF receptor family that do not comprise a death domain.

In another embodiment of the invention, invention TPBDs have been found to bind to TRAF-associated proteins. For example, the TRAF-7 TPBD (SEQ ID NOS:12 or 25) binds I-TRAF. Further, TRAF proteins generally are known to interact with numerous TRAF-



associated proteins, such as TRADD, FADD, I-TRAF, TRIP, A20, c-IAP1, c-IAP2, Casper, RIP, RIP2, NIK, Peg3, GCK, NIK, ASK1 and IRAK. Thus, it is contemplated herein that TPBDs also bind one or more TRAF-associated proteins

5 selected from TRADD, FADD, I-TRAF, TRIP, A20, c-IAP1, c-IAP2, Casper, RIP, RIP2, NIK, Peg3, GCK, NIK, ASK1 and IRAK.

Structurally, an invention TPBD is characterized as having the sequence: E(X)<sub>17</sub>.

10 <sub>21</sub>LXW(X)<sub>3</sub>VXP(X)<sub>15-16</sub>L(X)<sub>24-26</sub>K(X)<sub>15-16</sub>W (SEQ ID NO:19), where X is any amino acid. Alternatively, an invention TRAF domain is characterized as having the sequence:

LXWX(X')XVXP (SEQ ID NO:20) where X is any amino acid and X' is selected from L and I. Preferably, an invention

15 TRAF domain has the sequence: E(X)<sub>10-13</sub>S(X)<sub>6-7</sub>LXW(X)<sub>3</sub>VXP(X)<sub>10-11</sub>S(X)<sub>4</sub>L(X)<sub>24-26</sub>K(X)<sub>9-10</sub>F(X)<sub>5</sub>WG(X)<sub>3</sub>F(X)<sub>16</sub>D(X)<sub>5-7</sub>V (SEQ ID NO:21), where X is any amino acid. More preferably, an invention TRAF domain has the following sequence: E(X)<sub>4</sub>(X<sub>A</sub>)(X)<sub>5-9</sub>S(X<sub>B</sub>)(X)<sub>4-5</sub>LXWX(X<sub>A</sub>)XVXP(X)<sub>10-11</sub>S(X<sub>A</sub>)(X)<sub>3</sub>L(X)<sub>16-18</sub>(X<sub>A</sub>)(X)<sub>4-6</sub>(X<sub>C</sub>)(X)<sub>2</sub>K(X)<sub>9-10</sub>F(X)<sub>5</sub>WG(X<sub>D</sub>)(X)<sub>2</sub>F(X)<sub>5</sub>(X<sub>A</sub>)X(X<sub>C</sub>)(X)<sub>7</sub>(X<sub>C</sub>)DX(X<sub>A</sub>)(X)<sub>2-4</sub>(X<sub>C</sub>)V (SEQ ID NO:22), where X is any amino acid, X<sub>A</sub> is selected from V, L and I; X<sub>B</sub> is selected from P and G; X<sub>C</sub> is selected from D, E, N and Q; and X<sub>D</sub> is selected from Y and F. Most preferably, an invention

20 TRAF domain comprises the sequence SEQ ID NOS:8, 10, 12, 23, 24 or 25.

TRAF domain proteins, generally, are well known in the art to modulate the activity of NF-κB and JNK. In accordance with another embodiment of the invention,

30 invention TPBDs, HAUSP (preferably SEQ ID NOS:8 or 23), SPOP (SEQ ID NOS:10 or 24), and TRAF-7 (SEQ ID NOS:12 or

25), have been found to modulate the activity NF- $\kappa$ B or cJun N-terminal kinase (JNK).

It has also been found that invention TPBDs modulate a variety of cellular pathways. TRAF domain proteins, generally, are well known in the art as modulating the cellular pathways that effect apoptosis, cell proliferation, cell adhesion, cell stress responses and B cell immunoglobulin class switching, and NF- $\kappa$ B and JNK are further known to modulate these pathways. Thus, those of skill in the art will recognize that it is within the scope of the invention that TPBDs modulate one or more cellular pathways that effect apoptosis, cell proliferation, cell adhesion, cell stress responses and B cell immunoglobulin class switching.

Presently preferred TRAF domains of the invention include amino acid sequences that comprise substantially the same protein sequence set forth in SEQ ID NOS:8, 10, 12, 23, 24 and 25, as well as biologically active, modified forms thereof.

In another embodiment, invention TPBDs include proteins comprising TPBD fragments having the sequence SEQ ID NO:19 or SEQ ID NO:20, which retain at least one native biological TPBD activity, such as immunogenicity, the ability to bind to a TNF family receptor, the ability to bind a TRAF domain protein, the ability to bind a TRAF-associated protein, the ability to modulate NF- $\kappa$ B activity or JNK activity, the ability to modulate apoptosis, cell proliferation, cell adhesion, cell stress responses or B cell immunoglobulin class switching, provided the TRAF protein is no longer than 213 amino acids.

Use of the terms "isolated" and/or "purified" in the present specification and claims as a modifier of DNA, RNA, polypeptides or proteins means that the DNA, RNA, polypeptides or proteins so designated have been produced in such form by the hand of man, and thus are separated from their native *in vivo* cellular environment, and are substantially free of any other species of nucleic acid or protein. As a result of this human intervention, the recombinant DNAs, RNAs, polypeptides and proteins of the invention are useful in ways described herein that the DNAs, RNAs, polypeptides or proteins as they naturally occur are not.

As used herein, "eukaryotic" refers to the variety of species from which an invention TPBD is derived, e.g., yeast, slime mold, plant, insect, nematode, mammal, and the like. A preferred TPBD herein, is mammalian TRAF.

As used herein, "mammalian" refers to the variety of species from which a preferred invention TPBD is derived, e.g., human, rat, mouse, rabbit, monkey, baboon, bovine, porcine, ovine, canine, feline, and the like. A more preferred TPBD herein, is human TRAF.

The term "biologically active" or "functional", when used herein as a modifier of invention TPBD(s), or polypeptide fragment thereof, refers to a polypeptide that exhibits functional characteristics similar to a TPBD. For example, one biological activity of a TPBD is the ability to bind, preferably *in vivo*, to a TRAF protein. Such TRAF binding activity can be assayed, for example, using the methods described in the Examples described herein.

Another biological activity of TRAF is the ability to act as an immunogen for the production of polyclonal and monoclonal antibodies that bind specifically to an invention TPBD. Thus, an invention

- 5 TPBD will encode a polypeptide specifically recognized by an antibody that also specifically recognizes the TPBD having the amino acid sequence SEQ ID NO:19 or SEQ ID NO:20, preferably including an amino acid set forth in SEQ ID NOS:8, 10, 12, 23, 24 or 25. Such immunologic
- 10 activity may be assayed by any method known to those of skill in the art. For example, a test-TPBD polypeptide can be used to produce antibodies, which are then assayed for their ability to bind to an invention TPBD comprising SEQ ID NO:19 or SEQ ID NO:20, preferably including a
- 15 sequence set forth in SEQ ID NOS:8, 10, 12, 23, 24 or 25. If the antibody binds to the test-polypeptide and a protein including the sequence SEQ ID NO:19 or SEQ ID NO:20, and preferably also a protein including SEQ ID NOS:8, 10, 12, 23, 24 or 25 with substantially the same
- 20 affinity, then the polypeptide possesses the requisite immunologic biological activity.

- The TPBD containing protein set forth in SEQ ID NO:2 was initially identified as a herpes-associated ubiquitin-specific protease (HAUSP) associated with the
- 25 nuclear domain of the PML protein and with Herpes simplex virus type 1 protein Vmw110 (Everett et al., EMBO J. 16:566-577 (1997)). The subject application represents the first identification of a portion of this protein as forming a TRAF domain.

- 30 The TPBD containing protein set forth in SEQ ID NO:4 was initially identified in serum from a scleroderma patient as having a speckled pattern in nuclei, and was termed a speckle-type POZ protein, or SPOP (Nagai et al.,

FEBS Lett. 418:23-26 (1997)). The POZ domain of SPOP has an undetermined function, but the protein was found to be widely expressed.

The TPBD containing protein set forth in SEQ ID NO:6 was identified as having a leucine-zipper region, a zinc finger region and a leucine zipper-like K-box region (Nagase et al., DNA Res. 5:355-364 (1998)). Initially named KIAA0898, this protein is also referred to herein as TRAF7.

In addition to the TRAF domain, TRAF7 was also found to have a number of previously unrecognized domains (see Figure 3). The existence of a RING finger domain (amino acids 15-55) close to the N-terminus of TRAF7 was previously known. TRAF7 is KIAA0898, which was described in the database as a RING finger protein of unknown function. Besides this domain, several other new protein domains were identified in this molecule. The RING finger domain is followed by a ZF-BBox domain (amino acids 90-132) and a coiled coil (amino acids 132-177). These three protein domains form what is denominated the tripartite motif. The tripartite motif has been found in a very restricted list of proteins, including transcription factors, ribonucleoproteins, and oncoproteins and appears to be involved in protein-protein interaction (Borden, Biochem. Cell. Biol. 76:351-358 (1998)). After the tripartite motif, a second coiled coil (amino acids 195-231) is found. Two putative leucine zipper domains are also found in this region of the protein (amino acids 197-218 and 222-245). The TRAF domain (amino acids 277-403) is located after the leucine zippers. The TRAF domain is followed by another coiled coil (amino acids 427-446) and by two regions rich in acidic residues (amino acids 452-577 and 868-964).

Therefore, in addition to TRAF domains, including the TRAF domain of TRAF7 (SEQ ID NOS:12 or 25), the invention also provides additional functional domains of TRAF7, including a ZF-BBox domain (amino acids 90-132 of SEQ ID NO:32); coiled coil domains (amino acids 132-177, 195-231 and 427-446 of SEQ ID NO:32); leucine zipper domains (amino acids 197-218 and 222-245 of SEQ ID NO:32); and poly-acidic domains (amino acids 452-577 and 868-964 of SEQ ID NO:32).

10 In accordance with one embodiment of the invention, it has been found that the invention TPBD (SEQ ID NOS:8 or 23) can bind all known human TRAF proteins (i.e., TRAF proteins 1 through 6), although the TRAF domain of HAUSP (SEQ ID NOS:8 or 23) binds TRAF 3 somewhat weakly, and binds TRAF 6 quite strongly. The invention TPBD of HAUSP (SEQ ID NOS:8 or 23) has also been found to inhibit NF- $\kappa$ B activation mediated by any of TRAF2, TRAF5 or TRAF6. The TPBD of TRAF7 (SEQ ID NOS:12 or 25) also was found to inhibit NF $\kappa$ B activation mediated by TRAF2 or TRAF6 (see Examples).

In another embodiment of the invention, the invention TPBD of SPOP (SEQ ID NOS:10 or 24) shows selectivity in TRAF protein interaction, binding human TRAF 1 and TRAF 6, but not human TRAF proteins 2, 3, 4 or 5. The invention TPBD of SPOP (SEQ ID NOS:10 or 24) has also been found to be specific in TNF-receptor interaction, binding TNF-receptor 2, but not FAS, CD40, lymphotoxin- $\beta$  receptor, NGF receptor and DR4. The invention TPBD of SPOP (SEQ ID NOS:10 or 24) has also found to inhibit NF- $\kappa$ B activation mediated by TRAF6.

In a further embodiment of the invention, the invention TPBD of TRAF7 (SEQ ID NOS:12 or 25) binds all

known human TRAF proteins, but binds TRAF 2 somewhat weakly, and binds TRAF 6 quite strongly. The invention TPBD of SPOP (SEQ ID NOS:10 or 24) also binds numerous members of the TNF-receptor family, including TNF-  
 5 receptor 2, CD40 (albeit weakly), lymphotoxin- $\beta$  receptor, NGF receptor and DR4, but not to FAS. The invention TPBD of SPOP (SEQ ID NOS:10 or 24) has also been demonstrated to inhibit NF- $\kappa$ B activation by either TRAF2 or TRAF6.

Those of skill in the art will recognize that  
 10 numerous residues of the above-described sequences can be substituted with other, chemically, sterically and/or electronically similar residues without substantially altering the biological activity of the resulting receptor species. In addition, larger polypeptide  
 15 sequences containing substantially the same sequence as amino acids set forth in SEQ ID NOS:8, 10, 12, 23, 24 and 25 therein (e.g., splice variants) are contemplated.

As employed herein, the term "substantially the same amino acid sequence" refers to amino acid sequences  
 20 having at least about 70% identity with respect to the reference amino acid sequence, and retaining comparable functional and biological activity characteristic of the protein defined by the reference amino acid sequence. Preferably, proteins having "substantially the same amino  
 25 acid sequence" will have at least about 80%, more preferably 90% amino acid identity with respect to the reference amino acid sequence; with greater than about 95% amino acid sequence identity being especially preferred. It is recognized, however, that polypeptides  
 30 (or nucleic acids referred to hereinbefore) containing less than the described levels of sequence identity arising as splice variants or that are modified by conservative amino acid substitutions, or by substitution

of degenerate codons are also encompassed within the scope of the present invention. Identity of any two amino acid sequences can be determined by those skilled in the art based, for example, on a BLAST 2.0 computer alignment, using default parameters.

The invention TPBDs can be isolated by a variety of methods well-known in the art, e.g., recombinant expression systems described herein, precipitation, gel filtration, ion-exchange, reverse-phase and affinity chromatography, and the like. Other well-known methods are described in Deutscher et al., Guide to Protein Purification: Methods in Enzymology Vol. 182, (Academic Press, (1990)), which is incorporated herein by reference. Alternatively, the isolated polypeptides of the present invention can be obtained using well-known recombinant methods as described, for example, in Sambrook et al., supra., 1989).

An example of the means for preparing the invention TPBD(s) is to express nucleic acids encoding the TPBD in a suitable host cell, such as a bacterial cell, a yeast cell, an amphibian cell (i.e., oocyte), or a mammalian cell, using methods well known in the art, and recovering the expressed polypeptide, again using well-known methods. Invention polypeptides can be isolated directly from cells that have been transformed with expression vectors as described below herein. The invention polypeptide, biologically functional fragments, and functional equivalents thereof can also be produced by chemical synthesis. For example, synthetic polypeptides can be produced using Applied Biosystems, Inc. Model 430A or 431A automatic peptide synthesizer (Foster City, CA) employing the chemistry provided by the manufacturer.



Also encompassed by the term TPBD are functional fragments or polypeptide analogs thereof. The term "functional fragment" refers to a peptide fragment that is a portion of a full length TPBD, provided that the portion has a biological activity, as defined above, that is characteristic of the corresponding full length protein. For example, a functional fragment of an invention TPBD can have an activity such as the ability, for example, to bind a TNF-family receptor, to bind another TRAF protein, to bind a TRAF-associated protein, or to modulate NF- $\kappa$ B activity or JNK activity, or to modulate the level of cell proliferation, apoptosis, cell adhesion, cell stress responses, class switching, and the like. In addition, the characteristic of a functional fragment of invention TPBDs to elicit an immune response is useful for obtaining an anti-TPBD antibodies. Thus, the invention also provides functional fragments of invention TPBDs, which can be identified using the binding and routine methods, such as bioassays described herein.

The term "polypeptide analog" includes any polypeptide having an amino acid residue sequence substantially the same as a sequence specifically shown herein in which one or more residues have been conservatively substituted with a functionally similar residue and which displays the ability to functionally mimic a TPBD as described herein. Examples of conservative substitutions include the substitution of one non-polar (hydrophobic) residue such as isoleucine, valine, leucine or methionine for another, the substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, between glycine and serine, the substitution of one basic residue such as lysine,

arginine or histidine for another, or the substitution of one acidic residue, such as aspartic acid or glutamic acid for another.

The amino acid length of functional fragments  
 5 or polypeptide analogs of the present invention can range from about 5 amino acids up to one residue less than a full-length protein sequence of an invention TPBD. In certain embodiments, the amino acid lengths include, for example, at least about 10 amino acids, at least about  
 10 20, at least about 30, at least about 40, at least about 50, at least about 75, at least about 100, at least about 150, at least about 200, at least about 213, at least about 250, at least about 300, at least about 350 or more amino acids in length up to one residue less than a full-length  
 15 TPBD-containing protein sequence, provided as sequence SEQ ID NO:2, 4 or 6.

Preferably, a fragment comprises a sequence selected from SEQ ID NO:19, 21 or 22. Such a fragment can also include at least about 10 residues at its amino-  
 20 terminus, carboxy-terminus, or both; at least about 20 residues at its amino-terminus, carboxy-terminus, or both; at least about 30 residues at its amino-terminus, carboxy-terminus, or both; at least about 40 residues at its amino-terminus, carboxy-terminus, or both; at least  
 25 about 50 residues at its amino-terminus, carboxy-terminus, or both; at least about 60 residues at its amino-terminus, carboxy-terminus, or both.

More preferably, a fragment comprises a sequence selected from SEQ ID NO:19, 21 or 22, further  
 30 comprising one or more domains selected from N-TRAF, Ring Finger, Zinc finger, coiled-coil, POZ, and USP. Most preferably, a fragment has at least one fewer domains

than the domains in proteins from SEQ ID NO:2, 4 or 6, wherein the domains are selected from N-TRAF, Ring Finger, Zinc finger, coiled-coil, POZ, and USP. Such domains are known in the art, as exemplified in Arch et al., supra.; and Wajant et al., supra. Identification of the domains in proteins from SEQ ID NO:2, 4 and 6 is carried out by reference to the publication reporting such proteins (i.e., Everett et al., supra., Nagai et al., supra., and Nagase et al., supra., for HAUSP (SEQ ID NO:2), SPOP (SEQ ID NO:4) and TRAF7 (SEQ ID NO:6), respectively).

As used herein the phrase "conservative substitution" also includes the use of a chemically derivatized residue in place of a non-derivatized residue, provided that such polypeptide displays the required binding activity. The phrase "chemical derivative" refers to a subject polypeptide having one or more residues chemically derivatized by reaction of a functional side group. Such derivatized molecules include, for example, those molecules in which free amino groups have been derivatized to form amine hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups, t-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups may be derivatized to form salts, methyl and ethyl esters or other types of esters or hydrazides. Free hydroxyl groups may be derivatized to form O-acyl or O-alkyl derivatives. The imidazole nitrogen of histidine may be derivatized to form N-im-benzylhistidine. Also included as chemical derivatives are those peptides which contain one or more naturally occurring amino acid derivatives of the twenty standard amino acids. For example: 4-hydroxyproline may be substituted for proline; 5-hydroxylysine may be substituted for lysine; 3-methylhistidine may be

substituted for histidine; homoserine may be substituted for serine; and ornithine may be substituted for lysine. Polypeptides of the present invention also include any polypeptide having one or more additions and/or deletions  
5 of residues, relative to the sequence of a polypeptide whose sequence is shown herein, so long as the required activity is maintained.

In accordance with another embodiment, novel TPBD-containing TRAF proteins are provided. Invention  
10 TPBD-containing TRAF proteins refer to a protein comprising an invention TPBD including SEQ ID NO:19 or including SEQ ID NO:20, or a recombinantly produced invention TPBD-containing TRAF protein, including naturally occurring allelic variants thereof encoded by  
15 mRNA generated by alternative splicing of a primary transcript, provided the TPBD-containing TRAF protein are not the sequence SEQ ID NO:2, 4 or 6. Preferably, a TPBD-containing TRAF protein comprises an invention TPBD with a sequence substantially the same as SEQ ID NO:8,  
20 10, 12, 23, 24, or 25. More preferably, a TPBD-containing TRAF protein comprises an invention TPBD with the sequence of SEQ ID NO:8, 10, 12, 23, 24, or 25.

A TPBD-containing TRAF protein comprising an invention TPBD domain is further characterized as binding  
25 one or more members of the TNF-family of receptors, or binding to one or more TRAF domain proteins, or binding to one or more TRAF associated proteins; or modulating NF-kB activity, or modulating cJun N-terminal kinase (JNK) activity; or modulating apoptosis, cell  
30 proliferation, cell adhesion, cell stress responses or B cell immunoglobulin class switching; or any combination thereof.

In another embodiment of the invention, TPBD-containing chimeric proteins are provided comprising an invention TPBD, or fragments thereof, having the sequence of SEQ ID NO:19 or SEQ ID NO:20, and further comprising

5 one or more sequences from a heterologous protein. Invention TPBD-containing chimeric proteins include, for example, polypeptides having the sequence SEQ ID NO:8, 10, 12, 23, 24 or 25. Sequences from heterologous proteins with which the TPBD or functional fragment

10 thereof are fused will include, for example, glutathione-S-transferase, an antibody, or other proteins or functional fragments thereof which facilitate recovery of the chimera. Further proteins with which the TPBD or functional fragment thereof are fused will include, for

15 example, luciferase, green fluorescent protein, an antibody, or other proteins or functional fragments thereof which facilitate identification of the chimera. Still further proteins with which the TPBD or functional fragment thereof are fused will include, for example, the

20 LexA DNA binding domain, ricin,  $\alpha$ -sarcin, an antibody, or other proteins which have therapeutic properties or other biological activity.

As such chimeric proteins include sequences from two different proteins, the resultant amino acid

25 sequence of the chimeric protein will typically be a non-naturally occurring sequence. Thus, in accordance with this embodiment of the invention, there are provided chimeric proteins comprising an invention TPBD, or fragments thereof, having the sequence of SEQ ID NO:19 or

30 SEQ ID NO:20, provided the sequence of the chimeric protein is not naturally occurring.

Further invention chimeric proteins contemplated herein are chimeric proteins wherein an

invention TPBD is combined with one or more domains selected from a Ring-Finger domain, a Zinc-finger domain and an N-TRAF domain from a heterologous protein. For example, the TPBD of SEQ ID NO:8, 10, 12, 23, 24, or 25 can be fused with the Ring-Finger domain of TRAF proteins such as human TRAF 2, 3, 4, 5 or 6, and the like. Another example of such a chimera is a protein wherein the TPBD of SEQ ID NO:8, 10, 12, 23, 24, or 25 is fused with the N-TRAF domain from a TRAF protein such as human TRAF 1, 2, 3, 4, 5 or 6, and the like.

Another embodiment of the invention provides TPBD, or a functional fragment thereof, fused with a moiety to form a conjugate. As used herein, a "moiety" can be a physical, chemical or biological entity which contributes functionality to TPBD or a functional fragment thereof. Functionalities contributed by a moiety include therapeutic or other biological activity, or the ability to facilitate identification or recovery of TPBD. Therefore, a moiety will include molecules known in the art to be useful for detection of the conjugate by, for example, by fluorescence, magnetic imaging, detection of radioactive emission, and the like. A moiety may also be useful for recovery of the conjugate, for example a His tag or other known tags used for protein isolation/purification, or a physical substance such as a bead. A moiety can be a therapeutic compound, for example, a cytotoxic drug which can be useful to effect a biological change in cells to which the conjugate localizes.

In accordance with another embodiment of the invention there are provided oligomers comprising invention TPBDs and fragments thereof, invention TPBD-containing proteins, TPBD-containing chimeric proteins,

or combinations thereof. It has been found that a TPBD such as the TPBD of TRAF 7 (SEQ ID NOS:12 or 25) can bind at least one other, equivalent TPBD in forming a homo-oligomer. Thus in one embodiment, the invention  
5 comprises homo-oligomers of invention TPBDs and fragments thereof, invention TPBD-containing proteins, TPBD-containing chimeric proteins, or combinations thereof.

In another embodiment of the invention, there are provided hetero-oligomers comprising invention TPBDs  
10 and fragments thereof, invention TPBD-containing proteins, TPBD-containing chimeric proteins, or combinations thereof. It was found that invention HAUSP-TPBD (SEQ ID NOS:8 or 23), SPOP-TPBD (SEQ ID NOS:10 or 24) and TRAF7-TPBD (SEQ ID NOS:12 or 25) all bind, for  
15 example, multiple TRAF proteins. Thus hetero-oligomers comprising invention TPBDs and fragments thereof, invention TPBD-containing proteins, TPBD-containing chimeric proteins, or combinations thereof, and further comprising TRAF proteins such as human TRAF1, TRAF2,  
20 TRAF3, TRAF4, TRAF5, TRAF6, or combinations thereof. For example, the HAUSP-TPBD (SEQ ID NOS:8 or 23) can form a hetero-oligomer with human TRAF1, TRAF2, TRAF3, TRAF4, TRAF5, TRAF6, or combinations thereof. In another  
example, the SPOP-TPBD (SEQ ID NOS:10 or 24) can form a  
25 hetero-oligomer with human TRAF1, TRAF6, or combinations thereof. In a further example, the TRAF7-TPBD (SEQ ID NOS:12 or 25) can form a hetero-oligomer with human TRAF1, TRAF2, TRAF3, TRAF4, TRAF5, TRAF6, or combinations thereof.

30 In accordance with another embodiment of the invention, there are provided isolated nucleic acids, which encode a novel TPBD, and fragments thereof, TPBD-containing TRAF proteins and TPBD-containing chimeric

proteins. Nucleic acids that encode a TPBD are those that encode a protein with the ability to bind, preferably *in vivo*, to one or more members of the Tumor Necrosis Factor Receptor-family (TNFR-family), or to one or more members of the TRAF family, or to one or more TRAF-associated proteins, or any combination thereof, or have the ability to modulate NF- $\kappa$ B activity, JNK activity, apoptosis, cell proliferation, cell adhesion, cell stress responses or B cell immunoglobulin class switching. An invention nucleic acid encodes a TPBD having the sequence:  $E(X)_{17-21}L(X)_2W(X)_3VXP(X)_{15-16}L(X)_{24-28}K(X)_{15-16}W$  (SEQ ID NO:19), where X is any amino acid. Alternatively, an invention nucleic acid encodes a TPBD having the sequence:  $LXWX(X')XVXP$  (SEQ ID NO:20) where X is any amino acid and X' is selected from L and I. Preferably, an invention nucleic acid encodes a TPBD having the sequence:  $E(X)_{10-13}S(X)_{6-7}LXW(X)_3VXP(X)_{10-12}S(X)_4L(X)_{24-28}K(X)_{9-10}F(X)_3WG(X)_3F(X)_{16}D(X)_{5-7}V$  (SEQ ID NO:21), where X is any amino acid. More preferably, an invention nucleic acid encodes a TPBD having the sequence:  $E(X)_4(X_A)(X)_{5-8}SX(X_B)(X)_{4-5}LXWX(X_A)XVXP(X)_{10-11}S(X_A)(X)_3L(X)_{16-18}(X_A)(X)_{4-6}(X_C)(X)_2K(X)_{9-10}F(X)_5WG(X_A)(X)_2F(X)_5(X_A)X(X_C)(X)_7(X_C)DX(X_A)(X)_{2-4}(X_C)V$  (SEQ ID NO:22), where X is any amino acid,  $X_A$  is selected from V, L and I;  $X_B$  is selected from P and G;  $X_C$  is selected from D, E, N and Q; and  $X_D$  is selected from Y and F. Most preferably, an invention nucleic acid encodes a TPBD comprising the sequence SEQ ID NO:8, 10, 12, 23, 24 or 25.

The nucleic acid molecules described herein are useful for producing invention proteins, when such nucleic acids are incorporated into a variety of protein expression systems known to those of skill in the art. In addition, such nucleic acid molecules or fragments thereof can be labeled with a readily detectable



substituent and used as hybridization probes for assaying for the presence and/or amount of an invention TPBD gene or mRNA transcript in a given sample. The nucleic acid molecules described herein, and fragments thereof, are also useful as primers and/or templates in a Polymerase Chain Reaction (PCR) for amplifying genes encoding invention proteins described herein.

The term "nucleic acid" (also referred to as polynucleotides) encompasses ribonucleic acid (RNA) or deoxyribonucleic acid (DNA), probes, oligonucleotides, and primers. DNA can be either complementary DNA (cDNA) or genomic DNA, e.g. a gene encoding a TPBD. One means of isolating a nucleic acid encoding a TPBD is to probe a mammalian genomic library with a natural or artificially designed DNA probe using methods well known in the art. DNA probes derived from the TPBD gene are particularly useful for this purpose. DNA and cDNA molecules that encode TPBDs can be used to obtain complementary genomic DNA, cDNA or RNA from eukaryotic (e.g., human, primate, mammal, plant, nematode, insect, yeast, and the like), or mammalian sources, or to isolate related cDNA or genomic clones by the screening of cDNA or genomic libraries, by methods described in more detail below. Examples of nucleic acids are RNA, cDNA, or isolated genomic DNA encoding a TPBD, provided the nucleic acids do not comprise the nucleotide sequence set forth in SEQ ID NOs:1, 3, or 5. Such nucleic acids may include, but are not limited to, nucleic acids comprising substantially the same nucleotide sequence as set forth in SEQ ID NOs:7, 9, and 11.

In one embodiment of the present invention, cDNAs encoding the invention TPBD disclosed herein comprise substantially the same nucleotide sequence as

set forth in SEQ ID NOs:19 or 20, provided they do not comprise the sequence set forth in SEQ ID NO: 1, 3 or 5. Preferably, cDNAs encoding the invention TPBDs disclosed herein comprise substantially the same nucleotide  
5 sequence as set forth in any of SEQ ID NOs:7, 9, and 11, provided they do not comprise the sequence set forth in SEQ ID NO: 1, 3 or 5. Preferred cDNA molecules encoding the invention proteins comprise the same nucleotide sequence as set forth in SEQ ID No: 7, 9 and 11.

10 In another embodiment of the present invention, cDNAs encoding the invention TPBDs disclosed herein comprise substantially the same nucleotide sequence as set forth in SEQ ID NOs:19 or 20, provided they are no longer than 639 bases in length. Preferably, cDNAs  
15 encoding the invention TPBDs disclosed herein comprise substantially the same nucleotide sequence as set forth in any of SEQ ID NOs:7, 9, and 11, provided they are not longer than 639 bases in length. Preferred cDNA molecules encoding the invention proteins comprise the  
20 same nucleotide sequence as set forth in SEQ ID No: 7, 9 and 11.

cDNA molecules SEQ ID NOs:7, 9 and 11 encoding the invention TRAF domains respectively represent the same nucleotide sequence as nucleotides 1-639 set forth  
25 in SEQ ID No:1; nucleotides 1-540 set forth in SEQ ID NO:3; and nucleotides 847-1305 set forth in SEQ ID NO:5.

As employed herein, the term "substantially the same nucleotide sequence" refers to DNA having sufficient identity to the reference polynucleotide, such that it  
30 will hybridize to the reference nucleotide under moderately stringent hybridization conditions. In one embodiment, DNA having substantially the same nucleotide

sequence as the reference nucleotide sequence encodes substantially the same amino acid sequence as that set forth in any of SEQ ID Nos:8, 10, 12, 23, 24 and 25, provided the DNA does not encode the sequence set forth in SEQ ID NO: 2, 4 or 6. In another embodiment, DNA having "substantially the same nucleotide sequence" as the reference nucleotide sequence has at least 60% identity with respect to the reference nucleotide sequence. DNA having at least 70%, more preferably at least 90%, yet more preferably at least 95%, identity to the reference nucleotide sequence is preferred. Identity of any two nucleic acid sequences can be determined by those skilled in the art based, for example, on a BLAST 2.0 computer alignment, using default parameters. BLAST 2.0 searching is available at <http://www.ncbi.nlm.nih.gov/gorf/bl2.html>., as described by Tatiana et al., FEMS Microbiol Lett. 174:247-250 (1999).

This invention also encompasses nucleic acids which differ from the nucleic acids shown in SEQ ID NOs:7, 9, and 11, but which have the same phenotype. Phenotypically similar nucleic acids are also referred to as "functionally equivalent nucleic acids". As used herein, the phrase "functionally equivalent nucleic acids" encompasses nucleic acids characterized by slight and non-consequential sequence variations that will function in substantially the same manner to produce the same protein product(s) as the nucleic acids disclosed herein. In particular, functionally equivalent nucleic acids encode polypeptides that are the same as those encoded by the nucleic acids disclosed herein or that have conservative amino acid variations. For example, conservative variations include substitution of a non-polar residue with another non-polar residue, or

substitution of a charged residue with a similarly charged residue. These variations include those recognized by skilled artisans as those that do not substantially alter the tertiary structure of the protein.

Further provided are nucleic acids encoding TPBDs that, by virtue of the degeneracy of the genetic code, do not necessarily hybridize to the invention nucleic acids under specified hybridization conditions. Preferred nucleic acids encoding the invention TPBDs are comprised of nucleotides that encode substantially the same amino acid sequence as set forth in SEQ ID NOS:8, 10, and 12, provided they do not encode the sequence set forth in SEQ ID NO: 2, 4 or 6.

Thus, an exemplary nucleic acid encoding an invention TPBD may be selected from:

- (a) DNA encoding the amino acid sequence set forth in SEQ ID NOS:19 or 20
- (b) DNA that hybridizes to the DNA of (a) under moderately stringent conditions, wherein said DNA encodes biologically active TPBD, or
- (c) DNA degenerate with (b), wherein said DNA encodes biologically active TPBD, wherein the nucleic acid sequence does not encode an amino acid sequence longer than 213 residues.

Another exemplary nucleic acid encoding an invention TPBD may be selected from:

- (a) DNA encoding the amino acid sequence set forth in SEQ ID NOS:8, 10, 12, 23, 24 and 25,
- (b) DNA that hybridizes to the DNA of (a) under moderately stringent conditions, wherein

said DNA encodes biologically active TPBD, or  
 (c) DNA degenerate with (b), wherein said  
 DNA encodes biologically active TPBD,  
 wherein the nucleic acid sequence does not encode the  
 5 amino acid sequence set forth in SEQ ID Nos:2, 4 or 6.

Hybridization refers to the binding of  
 complementary strands of nucleic acid (i.e.,  
 sense:antisense strands or probe:target-DNA) to each  
 other through hydrogen bonds, similar to the bonds that  
 10 naturally occur in chromosomal DNA. Stringency levels  
 used to hybridize a given probe with target-DNA can be  
 readily varied by those of skill in the art.

The phrase "stringent hybridization" is used  
 herein to refer to conditions under which polynucleic  
 15 acid hybrids are stable. As known to those of skill in  
 the art, the stability of hybrids is reflected in the  
 melting temperature ( $T_m$ ) of the hybrids. In general, the  
 stability of a hybrid is a function of sodium ion  
 concentration and temperature. Typically, the  
 20 hybridization reaction is performed under conditions of  
 lower stringency, followed by washes of varying, but  
 higher, stringency. Reference to hybridization  
 stringency relates to such washing conditions.

As used herein, the phrase "moderately  
 25 stringent hybridization" refers to conditions that permit  
 target-DNA to bind a complementary nucleic acid that has  
 about 60% identity, preferably about 75% identity, more  
 preferably about 85% identity to the target DNA; with  
 greater than about 90% identity to target-DNA being  
 30 especially preferred. Preferably, moderately stringent  
 conditions are conditions equivalent to hybridization in  
 50% formamide, 5X Denhart's solution, 5X SSPE, 0.2% SDS

at 42°C, followed by washing in 0.2X SSPE, 0.2% SDS, at 42°C.

The phrase "high stringency hybridization" refers to conditions that permit hybridization of only those nucleic acid sequences that form stable hybrids in 0.018M NaCl at 65°C (i.e., if a hybrid is not stable in 0.018M NaCl at 65°C, it will not be stable under high stringency conditions, as contemplated herein). High stringency conditions can be provided, for example, by hybridization in 50% formamide, 5X Denhart's solution, 5X SSPE, 0.2% SDS at 42°C, followed by washing in 0.1X SSPE, and 0.1% SDS at 65°C.

The phrase "low stringency hybridization" refers to conditions equivalent to hybridization in 10% formamide, 5X Denhart's solution, 6X SSPE, 0.2% SDS at 42°C, followed by washing in 1X SSPE, 0.2% SDS, at 50°C. Denhart's solution and SSPE (see, e.g., Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1989) are well known to those of skill in the art as are other suitable hybridization buffers.

As used herein, the term "degenerate" refers to codons that differ in at least one nucleotide from a reference nucleic acid, e.g., SEQ ID NOs:7, 9, and 11, but encode the same amino acids as the reference nucleic acid. For example, codons specified by the triplets "UCU", "UCC", "UCA", and "UCG" are degenerate with respect to each other since all four of these codons encode the amino acid serine.

Preferred nucleic acids encoding the invention polypeptide(s) hybridize under moderately stringent,

preferably high stringency, conditions to substantially the entire sequence, or substantial portions (i.e., typically at least 15-30 nucleotides) of the nucleic acid sequence set forth in SEQ ID NOs:7, 9, and 11, provided  
5 they do not comprise the sequence set forth in SEQ ID NO: 1, 3 or 5.

The invention nucleic acids can be produced by a variety of methods well-known in the art, e.g., the methods described herein, employing PCR amplification  
10 using oligonucleotide primers from various regions of SEQ ID NOs:7, 9, and 11, and the like.

In accordance with a further embodiment of the present invention, optionally labeled TRAF-encoding cDNAs, or fragments thereof, can be employed to probe  
15 library(ies) (e.g., cDNA, genomic, and the like) for additional nucleic acid sequences encoding novel eukaryotic TPBDs. Construction of suitable eukaryotic cDNA libraries is well-known in the art. Screening of such a cDNA library is initially carried out under low-  
20 stringency conditions, which comprise a temperature of less than about 42°C, a formamide concentration of less than about 50%, and a moderate to low salt concentration.

Presently preferred probe-based screening conditions comprise a temperature of about 37°C, a  
25 formamide concentration of about 20%, and a salt concentration of about 5X standard saline citrate (SSC; 20X SSC contains 3M sodium chloride, 0.3M sodium citrate, pH 7.0). Such conditions will allow the identification of sequences which have a substantial degree of  
30 similarity with the probe sequence, without requiring perfect homology. The phrase "substantial similarity" refers to sequences which share at least 50% homology.

Preferably, hybridization conditions will be selected which allow the identification of sequences having at least 70% homology with the probe, while discriminating against sequences which have a lower degree of homology with the probe. As a result, nucleic acids having substantially the same nucleotide sequence as SEQ ID NOs:1, 3, and 5, are obtained.

As used herein, a nucleic acid "probe" is single-stranded DNA or RNA, or analogs thereof, that has a sequence of nucleotides that includes at least 14, at least 20, at least 50, at least 100, at least 200, at least 300, at least 400, or at least 500 contiguous bases that are the same as (or the complement of) any contiguous bases set forth in any of SEQ ID NOs:7, 9, and 11. Preferred regions from which to construct probes include 5' and/or 3' coding regions of SEQ ID NOs:7, 9, and 11. In addition, the entire cDNA encoding region of an invention TRAF, or the entire sequence corresponding to SEQ ID NOs:7, 9, and 11, may be used as a probe. Probes may be labeled by methods well-known in the art, as described hereinafter, and used in various diagnostic kits.

As used herein, the terms "label" and "indicating means" in their various grammatical forms refer to single atoms and molecules that are either directly or indirectly involved in the production of a detectable signal. Any label or indicating means can be linked to invention nucleic acid probes, expressed proteins, polypeptide fragments, or antibody molecules. These atoms or molecules can be used alone or in conjunction with additional reagents. Such labels are themselves well-known in clinical diagnostic chemistry.



The labeling means can be a fluorescent labeling agent that chemically binds to antibodies or antigens without denaturation to form a fluorochrome (dye) that is a useful immunofluorescent tracer. A description of immunofluorescent analytic techniques is found in DeLuca, "Immunofluorescence Analysis", in Antibody As a Tool, Marchalonis et al., eds., John Wiley & Sons, Ltd., pp. 189-231 (1982), which is incorporated herein by reference.

- 10 In one embodiment, the indicating group is an enzyme, such as horseradish peroxidase (HRP), glucose oxidase, and the like. In another embodiment, radioactive elements are employed labeling agents. The linking of a label to a substrate, i.e., labeling of
- 15 nucleic acid probes, antibodies, polypeptides, and proteins, is well known in the art. For instance, an invention antibody can be labeled by metabolic incorporation of radiolabeled amino acids provided in the culture medium. See, for example, Galfre et al., Meth. Enzymol., 73:3-46 (1981). Conventional means of protein
- 20 conjugation or coupling by activated functional groups are particularly applicable. See, for example, Aurameas et al., Scand. J. Immunol., Vol. 8, Suppl. 7:7-23 (1978), Rodwell et al., Biotech., 3:889-894 (1984), and U.S.
- 25 Patent No. 4,493,795.

- In another embodiment of the invention, nucleic acids are provided encoding chimeric proteins comprising an invention TPBD, or fragment thereof, having the sequence of SEQ ID NO:19 or SEQ ID NO:20, and further
- 30 comprising one or more sequences from a heterologous protein. Functional fragments of TPBD include, for example, polypeptides having the sequence SEQ ID NO:8, 10, 12, 23, 24 or 25. Nucleic acids encoding proteins

with which the TPBD or functional fragment thereof are fused will also encode, for example, glutathione-S-transferase, an antibody, or other proteins or functional fragments thereof which facilitate recovery of the chimera. Nucleic acids of the invention can also encode proteins with which the TPBD or functional fragment thereof are fused, for example, luciferase, green fluorescent protein, an antibody, or other proteins or functional fragments thereof which facilitate identification of the chimera. Still further nucleic acids of the invention encode proteins with which the TPBD or functional fragment thereof are fused including, for example, the LexA DNA binding domain, ricin,  $\alpha$ -sarcin, an antibody, or other proteins which have therapeutic properties or other biological activity.

The present invention also provides compositions containing an acceptable carrier and any of an isolated, purified TPBD-containing protein or functional polypeptide fragments thereof, alone or in combination with each other. These polypeptides or proteins can be recombinantly derived, chemically synthesized or purified from native sources. As used herein, the term "acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as phosphate buffered saline solution, water and emulsions such as an oil/water or water/oil emulsion, and various types of wetting agents.

The TPBD compositions described herein can be used, for example, in methods for modulating the activity of members of the TNFR family. TNF family receptor binding is well known in the art as mediating the signal transduction activity of the receptor, and it is demonstrated herein that invention TPBDs can bind TNF

receptors. Thus it is within the scope of the present invention that a protein comprising the sequence SEQ ID NO:19 or 20, or a nucleic acid encoding a protein comprising the sequence SEQ ID NO:19 or 20, modulates the activity of one or more TNF family receptors.

In one embodiment, modulation of a member of the TNFR family will comprise the step of contacting a member of the TNFR family with a protein comprising the sequence SEQ ID NO:19 or 20. Preferably, the method comprises contacting a cell with a protein comprising the sequence of SEQ ID NO:8, 10, 12, 23, 24 or 25.

In another embodiment, modulation of a member of the TNFR family will comprise the step of contacting a member of the TNFR family with a nucleic acid encoding a protein comprising the sequence SEQ ID NO:19 or 20. Preferably, the method comprises contacting a cell with a nucleic acid encoding a protein comprising the sequence of SEQ ID NO:8, 10, 12, 23, 24 or 25.

In another embodiment, the TPBD compositions described herein can be used, for example, in methods for modulating the activity of TRAF domain containing proteins. Thus it is within the scope of the present invention that a protein comprising the sequence SEQ ID NO:19 or 20, or a nucleic acid encoding a protein comprising the sequence SEQ ID NO:19 or 20, modulates the activity of one or more TRAF domain containing proteins.

In one embodiment, modulation of a TRAF domain containing protein will comprise the step of contacting a TRAF domain containing protein with a protein comprising the sequence SEQ ID NO:19 or 20. Preferably, the method comprises contacting a cell with a protein comprising the

sequence of SEQ ID NO:8, 10, 12, 23, 24 or 25.

In another embodiment, modulation of a TRAF domain containing protein will comprise the step of contacting a TRAF domain containing protein with a nucleic acid encoding a protein comprising the sequence SEQ ID NO:19 or 20. Preferably, the method comprises contacting a cell with a nucleic acid encoding a protein comprising the sequence of SEQ ID NO:8, 10, 12, 23, 24 or 25.

10 In another embodiment, a TPBD comprising the sequence SEQ ID NO:19 or 20, or a nucleic acid encoding a protein comprising the sequence SEQ ID NO:19 or 20, modulates the activity of one or more TRAF-associated proteins. While some TRAF-associated proteins are known to modulate NF- $\kappa$ B activity, others are known to modulate cJun N-terminal kinase (JNK) activity, and still others are known to modulate the activity of other proteins. For example, c-IAP1 and c-IAP2 modulate caspase activity and thus influence apoptosis. Thus it is within the scope of the invention that an invention TRAF domain protein can modulate the activity of TRAF-associated proteins any protein with which TRAF-associated proteins are known to interact.

In one embodiment, modulation of a TRAF-associated protein will comprise the step of contacting a TRAF-associated protein with a protein comprising the sequence SEQ ID NO:19 or 20. Preferably, the method comprises contacting a cell with a protein comprising the sequence of SEQ ID NO:8, 10, 12, 23, 24 or 25.

30 In another embodiment, modulation of a TRAF-associated protein will comprise the step of contacting a

TRAF-associated protein with a nucleic acid encoding a protein comprising the sequence SEQ ID NO:19 or 20.

Preferably, the method comprises contacting a cell with a nucleic acid encoding a protein comprising the sequence  
5 of SEQ ID NO:8, 10, 12, 23, 24 or 25.

TPBD compositions can also be used, for example, in methods for modulating the activity of NF- $\kappa$ B and cJun N-terminal kinase (JNK). Proteins homologous to invention TPBDs, for example, human TRAF domain proteins,  
10 are well known in the art as modulating the activity of NF- $\kappa$ B and JNK, and it is further shown herein that SEQ ID NOS:8, 10, 12, 23, 24 and 25 can modulate NF- $\kappa$ B activity. Thus, in accordance with another embodiment of the invention, a protein comprising the sequence SEQ ID NO:19  
15 or 20, or a nucleic acid encoding a protein comprising the sequence SEQ ID NO:19 or 20, modulates the activity of NF- $\kappa$ B or JNK.

In one embodiment, modulation of NF- $\kappa$ B or JNK activity will comprise the step of contacting a cell  
20 containing NF- $\kappa$ B or JNK activity with a protein comprising the sequence SEQ ID NO:19 or 20. Preferably, the method comprises contacting a cell with a protein comprising the sequence of SEQ ID NO:8, 10, 12, 23, 24 or 25.

In another embodiment, modulation of NF- $\kappa$ B or JNK activity will comprise the step of contacting a cell  
25 containing NF- $\kappa$ B or JNK activity with a nucleic acid encoding a protein comprising the sequence SEQ ID NO:19 or 20. Preferably, the method comprises contacting a  
30 cell with a nucleic acid encoding a protein comprising the sequence of SEQ ID NO:8, 10, 12, 23, 24 or 25.

The functions of the invention TPBDs support the role of TRAFs in modulating cellular pathways that effect apoptosis, cell proliferation, cell adhesion, cell stress responses and B cell immunoglobulin class switching. Thus, in accordance with another embodiment of the invention, a protein comprising the sequence SEQ ID NO:19 or 20, or a nucleic acid encoding a protein comprising the sequence SEQ ID NO:19 or 20, modulates apoptosis, cell proliferation, cell adhesion, cell stress responses or B cell immunoglobulin class switching.

In one embodiment, modulation of apoptosis, cell proliferation, cell adhesion, cell stress responses or B cell immunoglobulin class switching will comprise the step of contacting a cell with a protein comprising the sequence SEQ ID NO:19 or 20, whereby apoptosis, cell proliferation, cell adhesion, cell stress responses or B cell immunoglobulin class switching is modulated. Preferably, the method comprises contacting a cell with a protein comprising the sequence of SEQ ID NO:8, 10, 12, 23, 24 or 25.

In another embodiment, modulation of apoptosis, cell proliferation, cell adhesion, cell stress responses or B cell immunoglobulin class switching will comprise the step of contacting a cell with a nucleic acid encoding a protein comprising the sequence SEQ ID NO:19 or 20, whereby apoptosis, cell proliferation, cell adhesion, cell stress responses or B cell immunoglobulin class switching is modulated. Preferably, the method comprises contacting a cell with a nucleic acid encoding a protein comprising the sequence of SEQ ID NO:8, 10, 12, 23, 24 or 25.

Also provided are antisense-nucleic acids having a sequence capable of binding specifically with full-length or any portion of an mRNA that encodes TPBD polypeptides so as to prevent translation of the mRNA.

- 5 The antisense-nucleic acid may have a sequence capable of binding specifically with any portion of the sequence of the cDNA encoding TPBD polypeptides. As used herein, the phrase "binding specifically" encompasses the ability of a nucleic acid sequence to recognize a complementary  
10 nucleic acid sequence and to form double-helical segments therewith via the formation of hydrogen bonds between the complementary base pairs. An example of an antisense-nucleic acid is an antisense-nucleic acid comprising chemical analogs of nucleotides.

- 15 Compositions comprising an amount of the antisense-nucleic acid, described above, effective to reduce expression of TPBD polypeptides by passing through a cell membrane and binding specifically with mRNA encoding TPBD polypeptides so as to prevent translation  
20 and an acceptable hydrophobic carrier capable of passing through a cell membrane are also provided herein. Suitable hydrophobic carriers are described, for example, in U.S. Patent Nos. 5,334,761; 4,889,953; 4,897,355, and the like. The acceptable hydrophobic carrier capable of  
25 passing through cell membranes may also comprise a structure which binds to a receptor specific for a selected cell type and is thereby taken up by cells of the selected cell type. The structure may be part of a protein known to bind to a cell-type specific receptor.

- 30 Antisense-nucleic acid compositions are useful to inhibit translation of mRNA encoding invention polypeptides. Synthetic oligonucleotides, or other antisense chemical structures are designed to bind to

mRNA encoding TPBD polypeptides and inhibit translation of mRNA and are useful as compositions to inhibit expression of TPBD associated genes in a tissue sample or in a subject.

5           In accordance with another embodiment of the invention, kits for detecting mutations, duplications, deletions, rearrangements and aneuploidies in TPBD genes comprising at least one invention probe or antisense nucleotide.

10           The present invention provides means to modulate levels of expression of TPBD polypeptides by employing synthetic antisense-nucleic acid compositions (hereinafter SANC) which inhibit translation of mRNA encoding these polypeptides. Synthetic oligonucleotides,  
15 or other antisense-nucleic acid chemical structures designed to recognize and selectively bind to mRNA, are constructed to be complementary to full-length or portions of a TPBD coding strand, including nucleotide sequences set forth in SEQ ID NOS:7, 9, and 11. The SANC  
20 is designed to be stable in the blood stream for administration to a subject by injection, or in laboratory cell culture conditions. The SANC is designed to be capable of passing through the cell membrane in order to enter the cytoplasm of the cell by virtue of  
25 physical and chemical properties of the SANC which render it capable of passing through cell membranes, for example, by designing small, hydrophobic SANC chemical structures, or by virtue of specific transport systems in the cell which recognize and transport the SANC into the  
30 cell. In addition, the SANC can be designed for administration only to certain selected cell populations by targeting the SANC to be recognized by specific cellular uptake mechanisms which bind and take up the



SANC only within select cell populations. In a particular embodiment the SANC is an antisense oligonucleotide.

For example, the SANC may be designed to bind  
5 to a receptor found only in a certain cell type, as discussed *supra*. The SANC is also designed to recognize and selectively bind to target mRNA sequence, which may correspond to a sequence contained within the sequences shown in SEQ ID NOs:7, 9, and 11. The SANC is designed  
10 to inactivate target mRNA sequence by either binding thereto and inducing degradation of the mRNA by, for example, RNase I digestion, or inhibiting translation of mRNA target sequence by interfering with the binding of translation-regulating factors or ribosomes, or inclusion  
15 of other chemical structures, such as ribozyme sequences or reactive chemical groups which either degrade or chemically modify the target mRNA. SANCs have been shown to be capable of such properties when directed against mRNA targets (see Cohen et al., TIPS, 10:435 (1989) and  
20 Weintraub, Sci. American, January (1990), pp.40; both incorporated herein by reference).

In accordance with yet another embodiment of the present invention, there is provided a method for the recombinant production of invention TPBDs by expressing  
25 the above-described nucleic acid sequences in suitable host cells. Recombinant DNA expression systems that are suitable to produce TPBDs described herein are well-known in the art. For example, the above-described nucleotide sequences can be incorporated into vectors for further  
30 manipulation. As used herein, vector (or plasmid) refers to discrete elements that are used to introduce heterologous DNA into cells for either expression or replication thereof.

Suitable expression vectors are well-known in the art, and include vectors capable of expressing DNA operatively linked to a regulatory sequence, such as a promoter region that is capable of regulating expression of such DNA. Thus, an expression vector refers to a recombinant DNA or RNA construct, such as a plasmid, a phage, recombinant virus or other vector that, upon introduction into an appropriate host cell, results in expression of the inserted DNA. Appropriate expression vectors are well known to those of skill in the art and include those that are replicable in eukaryotic cells and/or prokaryotic cells and those that remain episomal or those which integrate into the host cell genome.

As used herein, a promoter region refers to a segment of DNA that controls transcription of DNA to which it is operatively linked. The promoter region includes specific sequences that are sufficient for RNA polymerase recognition, binding and transcription initiation. In addition, the promoter region includes sequences that modulate this recognition, binding and transcription initiation activity of RNA polymerase. These sequences may be *cis* acting or may be responsive to *trans* acting factors. Promoters, depending upon the nature of the regulation, may be constitutive or regulated. Exemplary promoters contemplated for use in the practice of the present invention include the SV40 early promoter, the cytomegalovirus (CMV) promoter, the mouse mammary tumor virus (MMTV) steroid-inducible promoter, Moloney murine leukemia virus (MMLV) promoter, and the like.

As used herein, the term "operatively linked" refers to the functional relationship of DNA with regulatory and effector nucleotide sequences, such as

promoters, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes, binds to and transcribes the DNA.

As used herein, expression refers to the process by which polynucleic acids are transcribed into mRNA and translated into peptides, polypeptides, or proteins. If the polynucleic acid is derived from genomic DNA, expression may, if an appropriate eukaryotic host cell or organism is selected, include splicing of the mRNA.

Prokaryotic transformation vectors are well-known in the art and include pBlueskript and phage Lambda ZAP vectors (Stratagene, La Jolla, CA), and the like. Other suitable vectors and promoters are disclosed in detail in U.S. Patent No. 4,798,885, issued January 17, 1989, the disclosure of which is incorporated herein by reference in its entirety.

Other suitable vectors for transformation of *E. coli* cells include the pET expression vectors (Novagen, see U.S. patent 4,952,496), e.g., pET11a, which contains the T7 promoter, T7 terminator, the inducible *E. coli* lac operator, and the lac repressor gene; and pET 12a-c, which contain the T7 promoter, T7 terminator, and the *E. coli* ompT secretion signal. Another suitable vector is the pIN-IIIompA2 (see Duffaud et al., Meth. in Enzymology, 153:492-507, 1987), which contains the lpp promoter, the lacUV5 promoter operator, the ompA

secretion signal, and the lac repressor gene.

Exemplary, eukaryotic transformation vectors, include the cloned bovine papilloma virus genome, the cloned genomes of the murine retroviruses, and eukaryotic  
 5 cassettes, such as the pSV-2 gpt system (described by Mulligan and Berg, Nature Vol. 277:108-114 (1979)) the Okayama-Berg cloning system (Mol. Cell Biol. 2:161-170 (1982)), and the expression cloning vector described by Genetics Institute (Wong et al., Science 228:810-815  
 10 (1985)), are available which provide substantial assurance of at least some expression of the protein of interest in the transformed eukaryotic cell line.

Particularly preferred base vectors which contain regulatory elements that can be linked to the  
 15 invention TPBD-encoding DNAs for transfection of mammalian cells are cytomegalovirus (CMV) promoter-based vectors such as pcDNA1 (Invitrogen, San Diego, CA), MMTV promoter-based vectors such as pMAMNeo (Clontech, Palo Alto, CA) and pMSG (Pharmacia, Piscataway, NJ), and SV40  
 20 promoter-based vectors such as pSV $\beta$  (Clontech, Palo Alto, CA).

In accordance with another embodiment of the present invention, there are provided "recombinant cells" containing the nucleic acid molecules (i.e., DNA or mRNA)  
 25 of the present invention. Methods of transforming suitable host cells, preferably bacterial cells, and more preferably *E. coli* cells, as well as methods applicable for culturing said cells containing a gene encoding a heterologous protein, are generally known in the art.  
 30 See, for example, Sambrook et al., Molecular Cloning: A Laboratory Manual (2 ed.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA (1989).

Exemplary methods of transformation include, e.g., transformation employing plasmids, viral, or bacterial phage vectors, transfection, electroporation, lipofection, and the like. The heterologous DNA can optionally include sequences which allow for its extrachromosomal maintenance, or said heterologous DNA can be caused to integrate into the genome of the host (as an alternative means to ensure stable maintenance in the host).

Host organisms contemplated for use in the practice of the present invention include those organisms in which recombinant production of heterologous proteins has been carried out. Examples of such host organisms include bacteria (e.g., *E. coli*), yeast (e.g., *Saccharomyces cerevisiae*, *Candida tropicalis*, *Hansenula polymorpha* and *P. pastoris*; see, e.g., U.S. Patent Nos. 4,882,279, 4,837,148, 4,929,555 and 4,855,231), mammalian cells (e.g., HEK293, CHO and Ltk<sup>-</sup> cells), insect cells, and the like. Presently preferred host organisms are bacteria. The most preferred bacteria is *E. coli*.

In one embodiment, nucleic acids encoding the invention TPBDs can be delivered into mammalian cells, either *in vivo* or *in vitro* using suitable viral vectors well-known in the art. Suitable retroviral vectors, designed specifically for "gene therapy" methods, are described, for example, in WIPO publications WO 9205266 and WO 9214829, which provide a description of methods for efficiently introducing nucleic acids into human cells. In addition, where it is desirable to limit or reduce the *in vivo* expression of the invention TRAF, the introduction of the antisense strand of the invention nucleic acid is contemplated.

Viral based systems provide the advantage of being able to introduce relatively high levels of the heterologous nucleic acid into a variety of cells. Suitable viral vectors for introducing invention nucleic acid encoding an TPBD into mammalian cells (e.g., vascular tissue segments) are well known in the art. These viral vectors include, for example, Herpes simplex virus vectors (e.g., Geller et al., Science, 241:1667-1669 (1988)), Vaccinia virus vectors (e.g., Piccini et al., Meth. in Enzymology, 153:545-563 (1987); Cytomegalovirus vectors (Mocarski et al., in Viral Vectors, Y. Gluzman and S.H. Hughes, Eds., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1988, pp. 78-84), Moloney murine leukemia virus vectors (Danos et al., PNAS, USA, 85:6469 (1980)), adenovirus vectors (e.g., Logan et al., PNAS, USA, 81:3655-3659 (1984); Jones et al., Cell, 17:683-689 (1979); Berkner, Biotechniques, 6:616-626 (1988); Cotten et al., PNAS, USA, 89:6094-6098 (1992); Graham et al., Meth. Mol. Biol., 7:109-127 (1991)), adeno-associated virus vectors, retrovirus vectors (see, e.g., U.S. Patent 4,405,712 and 4,650,764), and the like. Especially preferred viral vectors are the adenovirus and retroviral vectors.

For example, in one embodiment of the present invention, adenovirus-transferrin/polylysine-DNA (TfAdpl-DNA) vector complexes (Wagner et al., PNAS, USA, 89:6099-6103 (1992); Curiel et al., Hum. Gene Ther., 3:147-154 (1992); Gao et al., Hum. Gene Ther., 4:14-24 (1993)) are employed to transduce mammalian cells with heterologous TPBD nucleic acid. Any of the plasmid expression vectors described herein may be employed in a TfAdpl-DNA complex.

As used herein, "retroviral vector" refers to the well-known gene transfer plasmids that have an expression cassette encoding an heterologous gene residing between two retroviral LTRs. Retroviral vectors typically contain appropriate packaging signals that enable the retroviral vector, or RNA transcribed using the retroviral vector as a template, to be packaged into a viral virion in an appropriate packaging cell line (see, e.g., U.S. Patent 4,650,764).

Suitable retroviral vectors for use herein are described, for example, in U.S. Patent 5,252,479, and in WIPO publications WO 92/07573, WO 90/06997, WO 89/05345, WO 92/05266 and WO 92/14829, incorporated herein by reference, which provide a description of methods for efficiently introducing nucleic acids into human cells using such retroviral vectors. Other retroviral vectors include, for example, the mouse mammary tumor virus vectors (e.g., Shackelford et al., PNAS, USA, **85:9655-9659** (1988)), and the like.

In accordance with yet another embodiment of the present invention, there are provided anti-TPBD antibodies having specific reactivity with one or more TPBD polypeptides of the present invention. Active fragments of antibodies are encompassed within the definition of "antibody". Invention antibodies can be produced by methods known in the art using invention polypeptides, proteins or portions thereof as antigens. For example, polyclonal and monoclonal antibodies can be produced by methods well known in the art, as described, for example, in Harlow and Lane, Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory (1988)), which is incorporated herein by reference. Invention polypeptides can be used as immunogens in generating such

antibodies. Alternatively, synthetic peptides can be prepared (using commercially available synthesizers) and used as immunogens. Amino acid sequences can be analyzed by methods well known in the art to determine whether

5 they encode hydrophobic or hydrophilic domains of the corresponding polypeptide. Altered antibodies such as chimeric, humanized, CDR-grafted or bifunctional antibodies can also be produced by methods well known in the art. Such antibodies can also be produced by

10 hybridoma, chemical synthesis or recombinant methods described, for example, in Sambrook et al., supra., and Harlow and Lane, supra. Both anti-peptide and anti-fusion protein antibodies can be used. (see, for example, Bahouth et al., Trends Pharmacol. Sci. 12:338

15 (1991); Ausubel et al., Current Protocols in Molecular Biology (John Wiley and Sons, NY (1989) which are incorporated herein by reference).

Antibody so produced can be used, inter alia, in diagnostic methods and systems to detect the level of

20 TPBD present in a mammalian, preferably human, body sample, such as tissue or vascular fluid. Such antibodies can also be used for the immunoaffinity or affinity chromatography purification of the invention TRAF. In addition, methods are contemplated herein for

25 detecting the presence of an invention TPBD either within a cell, or on the surface of a cell, comprising contacting the cell with an antibody that specifically binds to TPBD polypeptides, under conditions permitting binding of the antibody to the TPBD polypeptides,

30 detecting the presence of the antibody bound to the TPBD polypeptide, and thereby detecting the presence of invention polypeptides on the surface of the cell. With respect to the detection of such polypeptides, the antibodies can be used for *in vitro* diagnostic or *in vivo*



imaging methods.

Immunological procedures useful for *in vitro* detection of target TPBD polypeptides in a sample include immunoassays that employ a detectable antibody. Such  
 5 immunoassays include, for example, ELISA, Pandex microfluorimetric assay, agglutination assays, flow cytometry, serum diagnostic assays and immunohistochemical staining procedures which are well known in the art. An antibody can be made detectable by  
 10 various means well known in the art. For example, a detectable marker can be directly or indirectly attached to the antibody. Useful markers include, for example, radionucleotides, enzymes, fluorogens, chromogens and chemiluminescent labels.

15 Invention anti-TPBD antibodies are contemplated for use herein to modulate the activity of the TPBD polypeptide in living animals, in humans, or in biological tissues or fluids isolated therefrom. The term "modulate" refers to a compound's ability to  
 20 increase (e.g., via an agonist), decrease (e.g., via an antagonist), or otherwise modify (e.g., increasing a first TPBD activity while decreasing a second TPBD activity) the biological activity of an invention TPBD protein, such as TNFR family-binding, TRAF protein  
 25 binding activity, TRAF-associated protein binding activity, NF- $\kappa$ B modulating activity, JNK modulating activity, apoptosis modulating activity, cell proliferation modulating activity, cell adhesion, cell stress responses modulating activity, or B cell  
 30 immunoglobulin class switching modulating activity, and the like. Accordingly, compositions comprising a carrier and an amount of an antibody having specificity for TPBD polypeptides effective to block naturally occurring

ligands or other TPBD-associated proteins, and the like, from binding to invention TPBD polypeptides are contemplated herein. For example, a monoclonal antibody directed to an epitope of an invention TPBD polypeptide including an amino acid sequence set forth in SEQ ID NOS:8, 10, 12, 23, 24 and 25 can be useful for this purpose.

The present invention further provides transgenic non-human mammals that are capable of expressing exogenous nucleic acids encoding TPBDs. As employed herein, the phrase "exogenous nucleic acid" refers to nucleic acid sequence which is not native to the host, or which is present in the host in other than its native environment (e.g., as part of a genetically engineered DNA construct). In addition to naturally occurring levels of TPBD-containing proteins, invention TPBDs can either be overexpressed or underexpressed (such as in the well-known knock-out transgenics) in transgenic mammals.

Also provided are transgenic non-human mammals capable of expressing nucleic acids encoding TPBD polypeptides so mutated as to be incapable of normal activity, i.e., do not express native TPBD. The present invention also provides transgenic non-human mammals having a genome comprising antisense nucleic acids complementary to nucleic acids encoding TPBD polypeptides, placed so as to be transcribed into antisense mRNA complementary to mRNA encoding TPBD polypeptides, which hybridizes to the mRNA and, thereby, reduces the translation thereof. The nucleic acid may additionally comprise an inducible promoter and/or tissue specific regulatory elements, so that expression can be induced, or restricted to specific cell types. Examples

of nucleic acids are DNA or cDNA having a coding sequence substantially the same as the coding sequence shown in SEQ ID NOs:1, 3, and 5. An example of a non-human transgenic mammal is a transgenic mouse. Examples of  
 5 tissue specificity-determining elements are the metallothionein promoter and the L7 promoter.

Animal model systems which elucidate the physiological and behavioral roles of TPBD polypeptides are also provided, and are produced by creating  
 10 transgenic animals in which the expression of the TPBD polypeptide is altered using a variety of techniques. Examples of such techniques include the insertion of normal or mutant versions of nucleic acids encoding an TRAF polypeptide by microinjection, retroviral infection  
 15 or other means well known to those skilled in the art, into appropriate fertilized embryos to produce a transgenic animal. (See, for example, Hogan et al., Manipulating the Mouse Embryo: A Laboratory Manual (Cold Spring Harbor Laboratory, (1986)).

Also contemplated herein, is the use of  
 20 homologous recombination of mutant or normal versions of TPBD genes with the native gene locus in transgenic animals, to alter the regulation of expression or the structure of TPBD polypeptides (see, Capecchi et al.,  
 25 Science 244:1288 (1989); Zimmer et al., Nature 338:150 (1989); which are incorporated herein by reference). Homologous recombination techniques are well known in the art. Homologous recombination replaces the native (endogenous) gene with a recombinant or mutated gene to  
 30 produce an animal that cannot express native (endogenous) protein but can express, for example, a mutated protein which results in altered expression of TPBD polypeptides.

In contrast to homologous recombination, microinjection adds genes to the host genome, without removing host genes. Microinjection can produce a transgenic animal that is capable of expressing both  
 5 endogenous and exogenous TPBDs. Inducible promoters can be linked to the coding region of nucleic acids to provide a means to regulate expression of the transgene. Tissue specific regulatory elements can be linked to the coding region to permit tissue-specific expression of the  
 10 transgene. Transgenic animal model systems are useful for *in vivo* screening of compounds for identification of specific ligands, i.e., agonists and antagonists, which activate or inhibit protein responses.

Invention nucleic acids, oligonucleotides  
 15 (including antisense), vectors containing same, transformed host cells, polypeptides and combinations thereof, as well as antibodies of the present invention, can be used to screen compounds *in vitro* to determine whether a compound functions as a potential agonist or  
 20 antagonist to invention TPBDs. These *in vitro* screening assays provide information regarding the function and activity of invention TPBDs, which can lead to the identification and design of compounds that are capable of specific interaction with one or more types of  
 25 polypeptides, peptides or proteins.

An invention TPBD, as used herein, contains the sequence  $E(X)_{17-21}L(X)_2W(X)_3VXP(X)_{15-16}L(X)_{24-26}K(X)_{15-16}W$  (SEQ ID NO:19), where X is any amino acid. Alternatively, an invention TPBD is characterized as having the sequence:  
 30  $LXWX(X')XVXP$  (SEQ ID NO:20) where X is any amino acid and X' is selected from L and I. Preferably, an invention TPBD has the sequence:  $E(X)_{10-13}S(X)_6LXW(X)_3VXP(x)_{10-11}S(X)_4L(X)_{24-26}K(X)_{9-10}F(X)_5WG(X)_3F(X)_{16}D(X)_{5-7}V$  (SEQ ID NO:21),

where X is any amino acid. More preferably, an invention TPBD has the following sequence: E(X)<sub>4</sub>(X<sub>A</sub>)(X)<sub>5-8</sub>SX(X<sub>B</sub>)(X)<sub>4-5</sub>LXWX(X<sub>A</sub>)XVXP(X)<sub>10-11</sub>S(X<sub>A</sub>)(X)<sub>3</sub>L(X)<sub>16-18</sub>(X<sub>A</sub>)(X)<sub>4-6</sub>(X<sub>C</sub>)(X)<sub>2</sub>K(X)<sub>9-10</sub>F(X)<sub>5</sub>WG(X<sub>A</sub>)(X)<sub>2</sub>F(X)<sub>5</sub>(X<sub>A</sub>)X(X<sub>C</sub>)(X)<sub>7</sub>(X<sub>C</sub>)DX(X<sub>A</sub>)(X)<sub>2-4</sub>(X<sub>C</sub>)V (SEQ ID NO:22), where X is any amino acid, X<sub>A</sub> is selected from V, L and I; X<sub>B</sub> is selected from P and G; X<sub>C</sub> is selected from D, E, N and Q; and X<sub>D</sub> is selected from Y and F. Most preferably, an invention TPBD comprises the sequence SEQ ID NO:8, 10, 12, 23, 24 or 25.

- 10 By the known homology of invention TPBDs to known TRAF domains, it is within the scope of the invention that invention TPBD also have a role in cellular pathways that effect apoptosis, cell proliferation, cell adhesion, cell stress responses and B cell
- 15 immunoglobulin class switching. Thus, invention TPBDs also provide drug discovery targets for a broad variety of pathologies including autoimmunity, inflammation, allergy, allograft-rejection and sepsis, and for a broad variety of cancer pathologies, such as, gliomas,
- 20 carcinomas, sarcomas, melanomas, hamartomas and the like. In certain aspects of the invention, invention TRAF proteins, agonist or antagonists thereto, are used to treat autoimmunity, inflammation, allergy, allograft-rejection, sepsis, keratinocyte hyperplasia, neoplasia,
- 25 keloid, benign prostatic hypertrophy, inflammatory hyperplasia, fibrosis, smooth muscle cell proliferation in arteries following balloon angioplasty (restenosis), and the like. Exemplary cancer pathologies contemplated herein for treatment include, gliomas, carcinomas,
- 30 adenocarcinomas, sarcomas, melanomas, hamartomas, leukemias, lymphomas, and the like.

Also provided herein are methods of treating pathologies, said method comprising administering an

effective amount of an invention therapeutic composition. Such compositions are typically administered in a physiologically compatible composition.

Methods of treating pathologies of abnormal cell proliferation will include methods of modulating the activity of one or more oncogenic proteins, wherein the oncogenic proteins specifically interact with a TPBD. Methods of modulating the activity of such oncogenic proteins will include contacting the oncogenic protein with a substantially pure TPBD or an active fragment (i.e., oncogenic protein-binding fragment) thereof. This contacting will modulate the activity of the oncogenic protein, thereby providing a method of treating a pathology caused by the oncogenic protein. Further methods of modulating the activity of oncogenic proteins will include contacting the oncogenic protein with an agent, wherein the agent modulates the interactions between the TPBD and the oncogenic protein.

Methods of treating immune-based pathologies such as autoimmunity, inflammation, allergy, allograft rejection, and sepsis will include modulating the activity of one or more proteins that modulate immune response, wherein the protein that modulates immune response specifically interact with a TPBD. Methods of modulating the activity of such protein that modulates immune response will include contacting the protein that modulates immune response with a substantially pure TPBD or an active fragment (i.e., protein-binding fragment) thereof. This contacting will modulate the activity of the protein that modulates immune response, thereby providing a method of treating a pathology caused by the protein that modulates immune response. Further methods of modulating the activity of a protein that modulates

immune response will include contacting the protein that modulates immune response with an agent, wherein the agent modulates the interactions between the TPBD and the protein that modulates immune response.

5 Also contemplated herein, are therapeutic methods using invention pharmaceutical compositions for the treatment of pathological disorders in which there is too little cell division, such as, for example, bone marrow aplasias, immunodeficiencies due to a decreased  
10 number of lymphocytes, and the like. Methods of treating a variety of inflammatory diseases with invention therapeutic compositions are also contemplated herein, such as treatment of sepsis, fibrosis (e.g., scarring), arthritis, graft versus host disease, and the like.

15 The present invention also provides therapeutic compositions useful for practicing the therapeutic methods described herein. Therapeutic compositions of the present invention, such as pharmaceutical compositions, contain a physiologically compatible  
20 carrier together with an invention TPBD (or functional fragment thereof), a TPBD modulating agent, such as a compound (agonist or antagonist) identified by the methods described herein, or an anti-TPBD antibody, as described herein, dissolved or dispersed therein as an  
25 active ingredient. In a preferred embodiment, the therapeutic composition is not immunogenic when administered to a mammal or human patient for therapeutic purposes.

30 As used herein, the terms "pharmaceutically acceptable", "physiologically compatible" and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably

and represent that the materials are capable of administration to a mammal without the production of undesirable physiological effects such as nausea, dizziness, gastric upset, and the like.

5           The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well known in the art. Typically such compositions are prepared as injectables either as liquid solutions or suspensions; however, solid forms  
10   suitable for solution, or suspension, in liquid prior to use can also be prepared. The preparation can also be emulsified.

          The active ingredient can be mixed with excipients which are pharmaceutically acceptable and  
15   compatible with the active ingredient in amounts suitable for use in the therapeutic methods described herein. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like, as well as combinations of any two or more thereof. In addition, if  
20   desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and the like, which enhance the effectiveness of the active ingredient.

          The therapeutic composition of the present  
25   invention can include pharmaceutically acceptable salts of the components therein. Pharmaceutically acceptable nontoxic salts include the acid addition salts (formed with the free amino groups of the polypeptide) that are formed with inorganic acids such as, for example,  
30   hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, phosphoric acid, acetic acid, propionic acid, glycolic acid, lactic



acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, fumaric acid, anthranilic acid, cinnamic acid, naphthalene sulfonic acid, sulfanilic acid, and the like.

5               Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium hydroxide, ammonium hydroxide, potassium hydroxide, and the like; and organic bases such as mono-, di-, and tri-alkyl and -aryl amines (e.g., triethylamine, 10 diisopropyl amine, methyl amine, dimethyl amine, and the like) and optionally substituted ethanolamines (e.g., ethanolamine, diethanolamine, and the like).

Physiologically tolerable carriers are well known in the art. Exemplary liquid carriers are sterile 15 aqueous solutions that contain no materials in addition to the active ingredients and water, or contain a buffer such as sodium phosphate at physiological pH, physiological saline or both, such as phosphate-buffered saline. Still further, aqueous carriers can contain more 20 than one buffer salt, as well as salts such as sodium and potassium chlorides, dextrose, polyethylene glycol and other solutes.

Liquid compositions can also contain liquid phases in addition to and to the exclusion of water. 25 Exemplary additional liquid phases include glycerin, vegetable oils such as cottonseed oil, and water-oil emulsions.

As described herein, an "effective amount" is a predetermined amount calculated to achieve the desired 30 therapeutic effect, e.g., to modulate activity of an invention TPBD. The required dosage will vary with the

particular treatment and with the duration of desired treatment; however, it is anticipated that dosages between about 10 micrograms and about 1 milligram per kilogram of body weight per day will be used for

5 therapeutic treatment. It may be particularly advantageous to administer such compounds in depot or long-lasting form as discussed hereinafter. A therapeutically effective amount is typically an amount of an TPBD-modulating agent or compound identified herein

10 that, when administered in a physiologically acceptable composition, is sufficient to achieve a plasma concentration of from about 0.1  $\mu\text{g/ml}$  to about 100  $\mu\text{g/ml}$ , preferably from about 1.0  $\mu\text{g/ml}$  to about 50  $\mu\text{g/ml}$ , more preferably at least about 2  $\mu\text{g/ml}$  and usually 5 to 10

15  $\mu\text{g/ml}$ . Therapeutic invention anti-TPBD antibodies can be administered in proportionately appropriate amounts in accordance with known practices in this art.

In accordance with still another embodiment of the present invention, there are provided methods for

20 identifying compounds which bind to TPBD polypeptides. The invention proteins may be employed in a competitive binding assay. Such an assay can accommodate the rapid screening of a large number of compounds to determine which compounds, if any, are capable of binding to TPBDs.

25 Subsequently, more detailed assays can be carried out with those compounds found to bind, to further determine whether such compounds act as modulators, agonists or antagonists of invention TPBDs. Compounds that bind to and/or modulate invention TPBDs can be used to treat a

30 variety of pathologies mediated by invention TPBDs.

In another embodiment of the invention, there is provided a bioassay for identifying compounds which modulate the activity of invention TPBD polypeptides.

Invention TPBD polypeptides are known to influence the activities of, for example, NF- $\kappa$ B. Further, homologous TRAF polypeptides are known to influence the activities of, for example, NF- $\kappa$ B and cJun N-terminal kinase (JNK).

5 Thus a reporter gene construct to assay for NF- $\kappa$ B or JNK activity can be used to test invention TPBD activity (see Examples). According to this method, invention TPBD polypeptides are contacted with an "unknown" or test substance, the activity of the invention TPBD polypeptide  
10 is monitored subsequent to the contact with the "unknown" or test substance, and those substances which effect a resultant modulation of, for example, NF- $\kappa$ B activity or JNK activity are identified as functional ligands for TPBD polypeptides.

15 Alternative bioassays for identifying compounds which modulate the activity of invention TPBD polypeptides can be used which routinely are used to test for protein:protein interactions. Such bioassays include yeast two-hybrid assays, glutathione-S-transferase fusion  
20 protein binding assays, co-immunoprecipitation assays, and the like. Such assays are well known in the art and can be found in standard reference texts such as Sambrook et al., supra, and Current Protocols in Molecular Biology, supra.

25 In accordance with another embodiment of the present invention, transformed host cells that recombinantly express invention polypeptides can be contacted with a test compound, and the modulating effect(s) thereof can then be evaluated by comparing the  
30 TPBD-mediated response (e.g., via reporter gene expression) in the presence and absence of test compound, or by comparing the response of test cells or control

cells (i.e., cells that do not express TPBD polypeptides), to the presence of the compound.

As used herein, a compound or a signal that "modulates the activity" of invention TPBD polypeptides  
5 refers to a compound or a signal that alters the activity of TRAF polypeptides so that the activity of the invention polypeptide is different in the presence of the compound or signal than in the absence of the compound or signal. In particular, such compounds or signals include  
10 agonists and antagonists. An agonist encompasses a compound or a signal that activates TPBD protein expression. Alternatively, an antagonist includes a compound or signal that interferes with TPBD expression. Typically, the effect of an antagonist is observed as a  
15 blocking of agonist-induced protein activation. Antagonists include competitive and non-competitive antagonists. A competitive antagonist (or competitive blocker) interacts with or near the site specific for agonist binding. A non-competitive antagonist or blocker  
20 inactivates the function of the polypeptide by interacting with a site other than the agonist interaction site.

As understood by those of skill in the art, assay methods for identifying compounds that modulate  
25 TPBD activity generally require comparison to a control. One type of a "control" is a cell or culture that is treated substantially the same as the test cell or test culture exposed to the compound, with the distinction that the "control" cell or culture is not exposed to the  
30 compound. For example, in methods that use voltage clamp electrophysiological procedures, the same cell can be tested in the presence or absence of compound, by merely changing the external solution bathing the cell. Another

type of "control" cell or culture may be a cell or culture that is identical to the transfected cells, with the exception that the "control" cell or culture do not express native proteins. Accordingly, the response of  
5 the transfected cell to compound is compared to the response (or lack thereof) of the "control" cell or culture to the same compound under the same reaction conditions.

In yet another embodiment of the present  
10 invention, the activation of TPBD polypeptides can be modulated by contacting the polypeptides with an effective amount of at least one compound identified by the above-described bioassays.

In accordance with another embodiment of the  
15 present invention, there are provided methods for diagnosing cancer, said method comprising:  
detecting, in said subject, a defective sequence or mutant of SEQ ID NOs:7, 9, and 11.

In accordance with another embodiment of the  
20 present invention, there are provided diagnostic systems, preferably in kit form, comprising at least one invention nucleic acid in a suitable packaging material. The diagnostic nucleic acids are derived from the TPBD-encoding nucleic acids described herein. In one  
25 embodiment, for example, the diagnostic nucleic acids are derived from any of SEQ ID NOs:7, 9, and 11. Invention diagnostic systems are useful for assaying for the presence or absence of nucleic acid encoding TPBD in either genomic DNA or in transcribed nucleic acid (such  
30 as mRNA or cDNA) encoding TRAF.

A suitable diagnostic system includes at least one invention nucleic acid, preferably two or more invention nucleic acids, as a separately packaged chemical reagent(s) in an amount sufficient for at least one assay. Instructions for use of the packaged reagent are also typically included. Those of skill in the art can readily incorporate invention nucleic probes and/or primers into kit form in combination with appropriate buffers and solutions for the practice of the invention methods as described herein.

As employed herein, the phrase "packaging material" refers to one or more physical structures used to house the contents of the kit, such as invention nucleic acid probes or primers, and the like. The packaging material is constructed by well known methods, preferably to provide a sterile, contaminant-free environment. The packaging material has a label which indicates that the invention nucleic acids can be used for detecting a particular sequence encoding TPBD including the nucleotide sequences set forth in SEQ ID NOs:7, 9, and 11 or mutations or deletions therein, thereby diagnosing the presence of, or a predisposition for, cancer. In addition, the packaging material contains instructions indicating how the materials within the kit are employed both to detect a particular sequence and diagnose the presence of, or a predisposition for, cancer.

The packaging materials employed herein in relation to diagnostic systems are those customarily utilized in nucleic acid-based diagnostic systems. As used herein, the term "package" refers to a solid matrix or material such as glass, plastic, paper, foil, and the like, capable of holding within fixed limits an isolated

nucleic acid, oligonucleotide, or primer of the present invention. Thus, for example, a package can be a glass vial used to contain milligram quantities of a contemplated nucleic acid, oligonucleotide or primer, or  
 5 it can be a microtiter plate well to which microgram quantities of a contemplated nucleic acid probe have been operatively affixed.

"Instructions for use" typically include a tangible expression describing the reagent concentration  
 10 or at least one assay method parameter, such as the relative amounts of reagent and sample to be admixed, maintenance time periods for reagent/sample admixtures, temperature, buffer conditions, and the like.

All U.S. patents and all publications mentioned  
 15 herein are incorporated in their entirety by reference thereto. The invention will now be described in greater detail by reference to the following non-limiting examples.

### EXAMPLES

#### I. Isolation of the TPBDs of TRAF7, HAUSP and SPOP

Nucleic acids encoding the TRAF protein binding domain fragments (TPBDs) of TRAF7 (residues 282-435 of SEQ ID NO:6), HAUSP (residues 1-213 of SEQ ID NO:2) and SPOP (residues 1-180 of SEQ ID NO:4) were isolated by  
 25 polymerase chain reaction (PCR) using 2 $\mu$ g of cDNA from Jurkat cells (The method for RNA extraction and cDNA synthesis was provided by the manufacturer (Pharmingen). The PCR was performed for 1 cycle of 94°C for 2 min followed by 35 cycles of 94°C for 15 s, 60°C for 20 s and  
 30 72°C for 100 s, followed by a final cycle of 72°C for 5

min, using the following primers HAUSP:

5' GCGAATTCCAGGCCGCG 3' (SEQ ID NO:13) and

5' TTCCTCGAGCCGACTTAGCCTGTGTGC 3' (SEQ ID NO:14); SPOP:

5' CTTCAATTTCGCGATGTCAAGGGTTCC 3' (SEQ ID NO:15) and

5' CCATGCTCGAGGTATTCTAGCCAGAAATG 3' (SEQ ID NO:16);

TRAF 7: 5' CCAGAATTCACCAAGTGAATTAGTGCC 3' (SEQ ID NO:17)

and 5' CCACTCGAGTAATGTACCAATGCTAGTGCC 3' (SEQ ID NO:18).

The amplified fragments were purified, digested with

EcoR1 and Xho 1 restriction enzymes and subcloned into

10 pCDNA-3-myc tag and into pGEX4T (Pharmacia).

## **II. Expression of TPBCs TRAF7, USP7 and SPOP**

To determine mRNA expression of genes encoding various TRAF domain proteins, Northern blot analysis of

the mRNA levels of TRAF7, USP7 and SPOP was performed in

15 human tissues. <sup>32</sup>P-labeled cDNA fragments of TRAF7, USP7

or SPOP corresponding to the TRAF domains were

sequentially hybridized, in the order of TRAF7, USP7 and

SPOP, to filter-immobilized poly-A<sup>+</sup> RNA from various

tissues (1 µg/lane) (Clontech; Palo Alto CA). The

20 tissues tested were brain, heart, skeletal muscle, colon,

thymus, spleen, kidney, liver, small intestine, placenta,

lung and peripheral blood leukocytes (PBL). Hybridized

fragments were visualized by autoradiography. The same

RNA blot was finally hybridized with a human β-actin cDNA

25 probe to control for RNA loading (bottom panel).

As shown in Figure 4, the TRAF7 probe

hybridized to mRNA of about 5 kb. TRAF7 mRNA was most

highly expressed in brain, heart, skeletal muscle,

kidney, liver and placenta. TRAF7 was expressed at a

30 lower level in thymus, small intestine, lung, spleen, PBL

and colon.



USP7, also known as HAUSP, was expressed as a doublet of about 7 and 5.5 kb. USP7 was most highly expressed in heart, skeletal muscle, and kidney, with lower expression in brain, thymus, liver, placenta and  
 5 PBLs, and lowest expression, barely detectable, in colon, spleen, small intestine and lung.

SPOP was expressed as mRNA of about 2.6 kb. SPOP was most highly expressed in heart and skeletal muscle, with lower expression in kidney and liver, and  
 10 even lower expression in brain, skeletal muscle, colon, thymus, small intestine, placenta, lung, and PBLs.

### III. Binding to the TNF-Receptor Family

For GST-fusion protein production, pGEX-plasmids were transformed into competent XL-1 blue  
 15 bacteria cells and grown in LB medium and induced at an  $A_{490} = 1.0$  with 1mM isopropyl-1-thio- $\beta$ -D-galactopyranoside for 4h at 25°C. Cells were then recovered and resuspended in PBS containing 1mM dithiothreitol, 1mM phenyl-methyl-sulphonyl-fluoride and 100  $\mu$ g/ml lysozyme  
 20 and lysed by sonication. The GST-TRAF2 (263-501), (GST-HAUSP (SEQ ID NO: 8), GST-SPOP (SEQ ID NO: 10) and GST-TRAF7 (SEQ ID NO: 12) proteins were purified from bacterial lysates by affinity chromatography using glutathione-Sepharose (Amersham Pharmacia Biotech). The  
 25 resins were then washed with PBS containing 1mM dithiothreitol until the  $OD_{280nm}$  reached  $<0.01$ .

Plasmids pGEX-TRAF2(263-501), pGEX-CD40(ct), pGEX-Fas(ct), pGEX-LTBR(ct), pGEX-DR4(ct) and pGEX-NGFR(ct) have been previously described (Leo et al.,  
 30 1999, JBC 274, 22414; Sato et al., 1995, FEBS lett. 358 113; Crowe et al., J Exp Med 1995, 181, 1205; McFarlane et

al., J Biol Chem 1997, 272,25417; Rabizadeh et al., Proc Natl Acad Sci U S A 1994, 91,10703). Plasmid pGEX-TNF-R2(ct) was kindly provided by Dr. C. Ware (La Jolla Institute for Allergy and Immunology).

- 5                    *In vitro* GST-protein binding assays were performed as previously described (Hanada, M., Aime-Sempe, C., Sato, T. and Reed, JC. 1995, JBC 270, 11962-11968; Sato, T., Irie, S., Kitada, S. and Reed, JC. 1995, Science 268, 411-415; Takayama, S., Sato, T., Krajewski, 10 S., Kochel, C., Irie, S., Millan, J. and Reed, JC. 1995, Cell 80, 279-284, Leo et al., 1999, JBC 274, 22414). Briefly, (<sup>35</sup>S)-methionine labeled GST-fused TNF family receptors FAS(ct), TNF-R2(ct), CD40(ct), LTBR(ct), NGFRp75(ct) and DR4(ct) were produced by *in vitro* 15 translation using the TnT coupled reticulocyte system following the manufacturer indications (Promega Inc.). Equal amounts of each labeled protein (2-6  $\mu$ l lysate) were then diluted with 250  $\mu$ l of binding buffer (142 mM KCl, 5 mM MgCl<sub>2</sub>, 10 mM Hepes pH 7.4, 0.2% Nonidet-P40, 20 0.5 mM dithiothreitol, 1mM EGTA, 0.5 mM phenyl-methylsulphonyl-fluoride and a mixture of other protease inhibitors (Boehringer 1697498) and incubated with the GST-protein resins (0.25 $\mu$ g protein) at 4°C for 2h. The resins were then extensively washed with binding buffer 25 and the GST-protein binding complexes were eluted with buffer containing 50 mM Tris-HCl pH 8, 1 mM dithiothreitol and 100 mM glutathione and analyzed by SDS-PAGE and fluorography.

- The results of the binding assay are shown in 30 Figure 5. *In vitro* protein binding assays were performed by *in vitro* translating TRAF2 or the TRAF domain of TRAF7 in the presence of <sup>35</sup>S-L-methionine in reticulocyte lysates, as described above, or following *in vivo*

overexpression of TRAF2 or the TRAF domain of TRAF7 by transfection of 293T cells. Equal volumes of the *in vitro* translation mixtures (10  $\mu$ l) were incubated with GST-fusion proteins containing the cytosolic domains of

5 different members of the TNF-R family (1  $\mu$ g) (FAS, TNF-R2, CD40, LT $\beta$ R, NGFRp75, and DR4) immobilized on glutathione-Sepharose. For TRAF domain protein expression *in vivo*, 293T lysates (50  $\mu$ l) were incubated with GST-fusion

10 proteins (1  $\mu$ g) containing the cytosolic domains of the TNF-R family members DR4, HVEM, TNF-R2, LT $\beta$ R, NGFRp75, CD40, and TRAF7 and then immobilized on glutathione-Sepharose. Control GST and other GST control proteins were included in all assays. After washing, bound

15 proteins were analyzed by SDS-PAGE followed by fluorescence for binding assays using *in vitro* translated proteins. For transfected 293 cells, samples were immunoblotted using anti-TRAF2 or anti-Myc antibodies to detect TRAF2 or TRAF7 TD binding, respectively. Bound

20 proteins were then detected by standard chemiluminescence assay (ECL; Amersham; Piscataway NJ).

As shown in Figure 5, three TNF family receptors interacted with the GST-fused TRAF domain of TRAF2: TNF-R2, CD40 and LT $\beta$ R. However, FAS, NGFRp75 and DR4 did not appreciably interact with the TRAF domain of

25 TRAF2. In contrast, the GST-SPOP fusion protein showed significant interaction with only one of these six receptors, TNF-R2; and GST-HAUSP did not significantly interact with any of the six receptors. In contrast, GST-TRAF7 binds TNF-R2, CD40 (albeit weakly), LT $\beta$ R,

30 NRFRp75 and DR4. This result shows that invention TPBDs SPOP (SEQ ID NO: 10) and HAUSP (SEQ ID NO: 8) can demonstrate higher selectivity in receptor binding than the TRAF domain of TRAF2 and the invention TPBD of TRAF7

(SEQ ID NO:12).

In cell lysates of 293 cells transfected with TRAF2 or the TRAF domain of TRAF7, binding was observed with several TNF-R family members. TRAF2 interacted with  
 5 HVEM, TNF-R2, LT $\beta$ R, CD40 and TRAF7, with low binding activity also observed with NGFRp75. The TRAF domain of TRAF7 interacted with DR4, LT $\beta$ R, and NGFRp75, with strongest binding observed with TRAF7. A faint band of TRAF7 was also observed to interact with TNF-R2 under  
 10 these conditions, but no binding of TRAF7 to CD40 or HVEM was detected. Therefore, TRAF7 has strong binding activity with itself.

#### IV. Binding to TRAF Family Proteins

The above described GST-HAUSP and GST-SPOP, and  
 15 GST-TRAF7 were produced and purified using the above described procedure.

Plasmids containing cDNAs encompassing the complete open reading frames of hTRAF 1 (pSG5-TRAF 1), hTRAF 2 (pcDNA3-HA-TRAF2), hTRAF3 (pbluscriptKS-TRAF3b),  
 20 hTRAF4 (pcDNA3-HA-TRAF4), hTRAF5 (pcDNA3-Flag-TRAF5) and hTRAF6 (pcDNA3-myc-TRAF6) have been previously described (Sato et al., 1995, FEBS lett.358, 113; Nakano et al., 1996, JBC 271, 14661; Mosialos et al., 1995, Cell 80, 389; Krajewska et al., 1998, Am.J.Pathol. 152, 1549; Leo  
 25 et al., 1999, JBC 274, 22414; Song and Donner.1995, Biochem. J. 309, 825; Rothe et al.,1995, Cell 78, 281).

In vitro translated TRAF1, TRAF2, TRAF3, TRAF4, TRAF5, TRAF6, full-length I-TRAF (Rothe et al., Proc. Natl. Acad. Sci. USA 93:8241-8246 (1996)), HAUSP (1-213,  
 30 SEQ ID NO:8), SPOP (1-180, SEQ ID NO:10) and TRAF7 (282-

435, SEQ ID NO:12) were incubated with the GST-protein resins using the previously described method.

The resultant images showed that the GST-fused TPBD of TRAF7 (SEQ ID NO: 12) interacted with all previously identified TRAF proteins, TRAFS1-6, and with I-TRAF (see Figure 6). Additionally, the TPBD of TRAF7 showed an ability to self-associate. The GST-fused TPBD of HAUSP (SEQ ID NO: 8) also was able to interact with all previously known TRAF proteins, although somewhat weakly with TRAF3, and weakly with I-TRAF. However, HAUSP did not demonstrate an ability to self-associate. In contrast, the GST-fused TPBD of SPOP (SEQ ID NO: 10) demonstrated a higher selectivity, having significant interactions with only TRAF1 and TRAF6, and no ability to self-associate. Interestingly, no heterologous interaction between invention TPBDs (i.e., HAUSP:SPOP, HAUSP:TRAF7 and SPOP:TRAF7) were observed.

## **V. Reporter Gene Assays**

293T cells were obtained from ATCC (Rockville, MA) and cultured in Dulbecco modified Eagle's-high glucose medium (Life technology, Inc) supplemented with 10% FCS (Hyclone, UT), 1mM glutamine and antibiotics. The promoter-containing reporter gene plasmids pUC13-4xNFkB-luc (containing 4 tandem HIV-NFkB response elements and the minimal fos promoter) and pCMV- $\beta$ -galactosidase has been previously described (Miyashita and Reed, 1995, Cell 80, 293 ; Lin and Stavnezer, 1996, MCB 16, 4591).

For NF-kB reporter gene assays, 293T cells were calcium phosphate-transfected with 12  $\mu$ g DNA, including

3.5 $\mu$ g of either pcDNA3-myc-hTRAF2, pcDNA3-myc-hTRAF5 or pcDNA3-myc-hTRAF6, in combination with either 3.5 or 7 $\mu$ g of control plasmid, pcDNA3-myc-HAUSP (1-213, SEQ ID NO: 8), pcDNA3-myc-SPOP (1-180, SEQ ID NO: 10) or pcDNA3-myc-TRAF7 (282-435, SEQ ID NO: 12) at 60% confluency in 6-well plates in duplicate, except in the case of TRAF5 in which only 7  $\mu$ g of pcDNA3-myc-HAUSP or pcDNA3-myc-TRAF7 were transfected. In the case of SPOP, only 3.5 $\mu$ g of either pcDNA3-myc-hTRAF6 was used. Also transfected were a total of 0.5 $\mu$ g pUC13-4 $\times$ NF $\kappa$ B-luc plasmid and 1  $\mu$ g pCMV- $\beta$ -galactosidase plasmid. After 36h, cells were lysed with 0.5 ml of Promega lysis buffer. The luciferase activity from 10  $\mu$ l of each cell lysate was determined using the Luciferase assay system from Promega, following the manufacturer protocol, and read using a luminometer (EG&G Berthold). The luciferase activity was normalized relative to  $\beta$ -galactosidase activity (Miyashita and Reed, 1995, Cell 80, 293).

Immunoblotting was performed as described (Krajewski et al., 1996, Anal. Biochem. 236, 221). Briefly, 5  $\mu$ l of each cell lysate was analyzed by immunoblotting using anti-TRAF6 or anti-myc antibodies and standard chemiluminiscence methods (Amersham) for detection.

The results show that TPBDs of HAUSP (SEQ ID NO: 8) and SPOP (SEQ ID NO: 10) and TRAF 7 (SEQ ID NO: 12) can inhibit NF- $\kappa$ B activation induced by TRAF6 (see Figure 7). In particular, the TPBD of HAUSP is capable of canceling nearly all of the TRAF6-induced increase in NF- $\kappa$ B activation. Further, the TPBD of HAUSP also strongly inhibits NF- $\kappa$ B activation induced by TRAF 2 and TRAF 5. The TPBD of TRAF 7 also demonstrates the ability

to inhibit TRAF 2-mediated NF- $\kappa$ B activation, but actually increases TRAF 5 mediated NF- $\kappa$ B activation.

The activity of various domains of the TRAF proteins USP7 and TRAF7 were further characterized with respect to regulating NF $\kappa$ B activity. The effect of TRAF7 and USP7 in regulating the NF $\kappa$ B activity induced by TRAF2 and TRAF6 in a mammalian cell line was tested. Briefly, 293T cells were transfected in 6 well plates with approximately 10  $\mu$ g of pcDNA3 control plasmid, 3.5  $\mu$ g of either pcDNA3-myc-hTRAF6 or pcDNA3-hTRAF2, and 7  $\mu$ g of any of several TRAF7 or USP7 plasmid constructs containing various domains (see Figure 8, bottom). The transfections also included 0.5  $\mu$ g pUC13-4 $\times$ NF $\kappa$ B-luc plasmid and 1  $\mu$ g pCMV- $\beta$ -galactosidase plasmid. Relative NF $\kappa$ B activity was assessed by luciferase assays using 10  $\mu$ l of each of the cell lysates prepared 36 h after transfection, with normalization for  $\beta$ -galactosidase activity. At least three to eight independent experiments were performed. The results are presented as fold of activation, with one representative experiment shown (Figure 8).

As shown in Figure 8, full length USP7 and a USP7 construct lacking the TRAF domain had a slight inhibitory effect on NF $\kappa$ B activity induced by TRAF2 or TRAF6. The USP7 TRAF domain alone strongly inhibited the TRAF2 and TRAF6-induced increase in NF $\kappa$ B activation. In contrast to USP7, full length TRAF7, as well as the TRAF domain of TRAF7 alone, had inhibitory activity for TRAF2 and TRAF6-induced increase in NF $\kappa$ B activation. A TRAF7 mutant construct lacking the TRAF domain did not show any effect on inhibiting the NF $\kappa$ B activation mediated by TRAF2 or TRAF6.

The effect of the TRAF domains of TRAF7, USP7 and SPOP in regulating the NFkB activity induced by TNF $\alpha$  or CD40 overexpression was also tested. 293T cells were transfected in 6 well plates with approximately 10  $\mu$ g of pcDNA3 control plasmid, 7  $\mu$ g of either TRAF7 TD, USP7 TD or SPOP TD plasmids, and either no plasmid (TNF $\alpha$  in Figure 9) or 3.5  $\mu$ g of pcDNA3-myc-CD40 (CD40 in Figure 9), together with 0.5  $\mu$ g pUC13-4xNFkB-luc plasmid and 1  $\mu$ g pCMV- $\beta$ -galactosidase plasmid. TNF $\alpha$  treatment was performed by adding 100 ng/ml of TNF $\alpha$  to the cells 12 h before harvesting. Relative NFkB activity was assessed by luciferase assays using 10  $\mu$ l of each of the cell lysates prepared 36 h after transfection, with normalization for  $\beta$ -galactosidase activity. At least three independent experiments were performed. The results of a representative experiment are presented as fold of activation.

As shown in Figure 9, the TRAF domain of TRAF7 had some inhibitory activity for activation of NFkB induced by both TNF $\alpha$  and CD40 overexpression. The TRAF domain of USP7 had even stronger inhibitory activity than the TRAF domain of TRAF7. In contrast, the TRAF domain of SPOP showed essentially no inhibitory activity of NFkB activation by either TNF $\alpha$  or CD40 overexpression.

Regulation by TRAF7 and USP7 of NFkB activation induced by the TRAF-associated protein NIK was also examined. 293T cells were transfected in 6 well plates with approximately 10  $\mu$ g of pcDNA3 control plasmid, 7  $\mu$ g of either empty pcDNA3, or pcDNA3-myc-containing TRAF7, TRAF7 TD, USP7 TD or SPOP TD plasmids, and 3.5  $\mu$ g of pcDNA3-Flag-NIK, together with 0.5  $\mu$ g pUC13-4xNFkB-luc plasmid and 1  $\mu$ g pCMV- $\beta$ -galactosidase plasmid. Relative NFkB activity was assessed by luciferase assays using 10



1 μl of each of the cell lysates prepared 36 h after  
 transfection, with normalization for β-galactosidase  
 activity. At least three independent experiments were  
 performed. The results of a representative experiment  
 5 are presented as fold of activation (Figure 10).

As shown in Figure 10, NIK induced NFκB  
 activation can be partially regulated by TRAF7 and USP7.  
 Both TRAF7 and the TRAF domain of TRAF7 partially  
 inhibited NIK-induced NFκB activation. USP7 TRAF domain  
 10 also partially inhibited NIK induced NFκB activation,  
 although the full length USP7 did not inhibit NIK-induced  
 NFκB activation (Figure 10)

#### **VI. Subcellular Localization of Domains of TPBDs**

To test the function of various domains in  
 15 subcellular localization, TRAF7 and USP7 deletion mutants  
 were constructed. For TRAF7 deletion mutants, Cos7 cells  
 were transfected with Lipofectamin plus (Life  
 Technologies; Rockville MD) and a total of 3 μg of  
 plasmid. The TRAF7 deletion mutants (shown in Figure 11)  
 20 were constructed as Myc fusion proteins. 24 hours after  
 transfection, cells were plated onto poly-lysinated  
 cover-glasses and allowed to settle for 24h more. Cells  
 were fixed with methanol-acetone (50% each). After  
 blocking, cells were stained with anti-myc mAb (Santa  
 25 Cruz Biotechnology; Santa Cruz CA) and a secondary FITC-  
 labelled rabbit anti-mouse Ig (Dako; Carpinteria CA).  
 Analysis was performed using a Bio-Rad confocal  
 microscope (BioRad; Hercules CA).

Figure 11 shows the subcellular localization of  
 30 TRAF7 and different deletion mutants of the molecule. A  
 scheme of the different TRAF7 mutants analyzed is also

shown. TRAF7 localizes in cytosolic corpuscles, which can appear as individual dots (panel A) or as large aggregates (A, small panel). Deletion mutants of TRAF7 lacking the RING finger domain (B), containing only the tripartite domain (C), lacking the poly-acidic region (D), or lacking the TRAF domain (E) have a similar subcellular localization as the full length TRAF7. In contrast, the TRAF domain alone (F) or the TRAF domain containing also the leucine zippers (G) did not show this particular subcellular localization, having a diffuse cytosolic localization. The C-terminal region of the molecule containing the TRAF domain and the poly-acidic region (H) or the poly-acidic region alone (I) have a similar diffuse cytosolic localization, although in this case they appear to be also located in the nucleus. These results indicate that the region of TRAF7 which is determinant for its subcellular localization can be mapped to the Z-b box and the first coiled coil.

For characterizing the localization of USP7 deletion mutants, Cos7 cells were transfected with Lipofectamin plus (Life Technologies) and a total of 3 µg of plasmid. USP7 full length and deletion mutants were constructed as Myc fusion proteins. The USP7 deletion mutants tested were a mutant lacking the TRAF domain and a mutant containing USP7 TRAF domain. 24 hours after transfection, cells were plated onto poly-lysinated cover-glasses and allowed to settle for 24h more. Cells were fixed with methanol-acetone (50% each). After blocking, cells were stained with anti-myc mAb (Santa Cruz Biothech.) and a secondary FITC-labelled rabbit anti-mouse Ig (Dako). Analysis was performed using a Bio-Rad confocal microscope. Two representative examples of each construct are shown.

Figure 12 shows the subcellular localization of USP7 full length (A and B), a deletion mutant of USP7 lacking the TRAF domain (C and D) and USP7 TRAF domain (E and F). USP7 is mainly located in the nucleus, although  
5 some cytosolic staining can also be observed in some cells. The deletion mutant lacking the TRAF domain is completely excluded from the nucleus, even when it still contains the nuclear localization signal. Finally, the TRAF domain of USP7 appears to have a diffuse  
10 localization, with a larger concentration in the perinuclear region. These results indicate that the TRAF domain of USP7 is necessary, but not sufficient, to target this molecule to the nucleus.

Although the invention has been described with  
15 reference to the examples above, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

That which is claimed is:

1. A TRAF-Protein-Binding-Domain (TPBD) polypeptide comprising SEQ ID NO:19, provided said polypeptide is no longer than 213 amino acids.

5           2. The TPBD polypeptide of claim 1, further comprising SEQ ID NO:20.

3. The TPBD polypeptide of claim 1, further comprising SEQ ID NO:21.

10           4. The TPBD of claim 1, wherein the amino acid sequence of said protein comprises substantially the same sequence as any of SEQ ID NOS:8, 10, 12, 23, 24 or 25.

15           5. The TPBD of claim 1, comprising the same amino acid sequence as set forth in any of SEQ ID NOS:8, 10, 12, 23, 24 or 25.

6. A TPBD according to claim 4, wherein said polypeptide is encoded by a nucleotide sequence comprising substantially the same nucleotide sequence as set forth in SEQ ID NOS:7, 9 or 11.

20           7. A TPBD according to claim 1, wherein said polypeptide is encoded by a nucleotide sequence comprising the same sequence as set forth in SEQ ID NOS:7, 9 or 11.

25           8. An isolated anti-TPBD antibody having specific reactivity with a TPBD according to claim 1.

9. Antibody according to claim 8, wherein said antibody is a monoclonal antibody.

10. A cell line producing the monoclonal antibody of claim 9.

5 11. An antibody according to claim 8, wherein said antibody is a polyclonal antibody.

12. Isolated nucleic acid encoding a TRAF protein binding domain (TPBD), or functional fragments thereof, selected from:

- 10 (a) DNA encoding the amino acid sequence set forth in SEQ ID NOS:8, 10 or 12, 23, 24 or 25, or
- (b) DNA that hybridizes to the DNA of (a) under moderately stringent conditions, wherein
- 15 said DNA encodes biologically active TPBD, or
- (c) DNA degenerate with (b), wherein said DNA encodes biologically active TPBD.

13. A nucleic acid according to claim 12, wherein said nucleic acid hybridizes under high

20 stringency conditions to the TPBD coding portion of any of SEQ ID NOS:7, 9 and 11.

14. A nucleic acid according to claim 12, wherein the nucleotide sequence of said nucleic acid is substantially the same as set forth in any of SEQ ID

25 NO:7, 9 and 11.

15. A nucleic acid according to claim 12, wherein the nucleotide sequence of said nucleic acid is the same as that set forth in any of SEQ ID NOS:7, 9 and 11.

16. A nucleic acid according to claim 12, wherein said nucleic acid is cDNA.

17. A vector containing the nucleic acid of claim 12.

18. Recombinant cells containing the nucleic acid of claim 12.

19. An oligonucleotide comprising at least 15 nucleotides capable of specifically hybridizing with a the nucleotide sequence set forth in any of SEQ ID NOs:7, 9 and 11.

20. An oligonucleotide according to claim 19, wherein said oligonucleotide is labeled with a detectable marker.

21. An antisense-nucleic acid capable of specifically binding to mRNA encoded by said nucleic acid according to claim 12.

22. A kit for detecting the presence of the TRAF cDNA sequence comprising at least one oligonucleotide according to claim 20.

23. A composition comprising an amount of the antisense-nucleic acid according to claim 21 effective to inhibit expression of a human TPBD and an acceptable hydrophobic carrier capable of passing through a cell membrane.

24. A method for expression of a TPBD, said method comprising culturing cells of claim 18 under conditions suitable for expression of said TPBD.

25. A method for identifying nucleic acids encoding a mammalian TPBD, said method comprising contacting a sample containing nucleic acids with an oligonucleotide according to claim 19, wherein said  
5 contacting is effected under high stringency hybridization conditions, and identifying compounds which hybridize thereto.

26. A method for detecting the presence of a human TPBD in a sample, said method comprising contacting  
10 a test sample with an antibody according to claim 8, detecting the presence of an antibody:TPBD complex, and therefor detecting the presence of a human TPBD in said test sample.

27. Single strand DNA primers for  
15 amplification of TPBD nucleic acid, wherein said primers comprise a nucleic acid sequence derived from the nucleic acid sequences set forth as SEQ ID NOs:7, 9 and 11.

28. A method of modulating a TNF family receptor comprising contacting a cell with an agent that  
20 modulates the activity of a TDBP-containing protein, wherein said TNF family receptor is selected from TNFR1, TNFR2, CD27, CD30, CD40, 4-1BB, Ox40, LT- $\beta$ R, Fas, DR3, DR4, DR5, HVEM, LMP-1, IL-1R, and a member of the TNF receptor family that does not comprise a death domain.

29. The method of claim 28 wherein said TNF  
25 family receptor is selected from TNF-R2, CD40, LT- $\beta$ R, NGFRp75 and DR4.

30. A method of modulating a TRAF protein comprising contacting a cell with an agent that modulates the activity of a TDBP-containing protein.

31. The method of claim 30 wherein said TRAF  
5 protein is selected from human TRAF1, human TRAF2, human TRAF3, human TRAF4, human TRAF5 and human TRAF6.

32. A method of modulating a TRAF-associated protein comprising contacting a cell with an agent that modulates the activity of a TDBP-containing protein,  
10 wherein said TRAF-associated protein is selected from TRADD, FADD, I-TRAF, TRIP, A20, c-IAP1, c-IAP2, Casper, RIP, RIP2, NIK, Peg3, GCK, NIK, ASK1 and IRAK.

33. A method of modulating the activity of NF-  
kB or cJun N-terminal kinase comprising contacting a cell  
15 with an agent that modulates the activity of a TDBP-containing protein.

34. The method of claim 33 wherein said NF-kB activity is modulated.

35. A method of modulating a cell process  
20 comprising contacting a cell with an agent that modulates the activity of a TDBP-containing protein, wherein said cell process is selected from apoptosis, cell proliferation, cell adhesion, cell stress responses and B cell immunoglobulin class switching.

25 36. A method for modulating the activity of an oncogenic protein, comprising contacting said oncogenic proteins with a substantially pure TPBD, or an oncogenic protein-binding fragment thereof.



37. A method of identifying an effective agent that modulates the association of a TPBD with a TRAF protein, comprising the steps of:

5 a) contacting said TPBD and TRAF proteins, under conditions that allow said TPBD and TRAF proteins to associate with an agent suspected of being able to modulate the association of said TPBD and TRAF proteins; and

10 b) detecting the modulated association of said TPBD and TRAF proteins, wherein said modulated association identifies an effective agent.

38. The method of claim 37, wherein said altered association is detected by measuring the activity  
15 of NF- $\kappa$ B.

39. The method of claim 37, wherein said altered association is detected by measuring the activity of c-Jun N-terminal kinase.

40. The method of claim 37, wherein said  
20 effective agent is a drug.

41. The method of claim 37, wherein said effective agent is a protein.

42. A method for modulating an activity mediated by a TPBD, said method comprising contacting  
25 said TPBD with an effective, modulating amount of an agent identified by claim 37.

43. The method of claim 42, wherein said modulated activity is selected from the group consisting of: binding of TPBD to a TNRF; binding of TPBD to a TRAF protein; binding of TPBD to a TRAF-associated protein; 5 NF- $\kappa$ B activity, c-Jun N-terminal kinase activity, apoptosis activity, cell proliferation activity, cell adhesion, cell stress response activity and B cell immunoglobulin class switching activity.

44. A method of modulating the level apoptosis 10 in a cell, comprising the steps of:

a) introducing a nucleic acid molecule encoding a TPBD into the cell; and

b) expressing said TPBD in said cell, wherein the expression of said TPBD modulates apoptosis 15 in said cell.

45. A method of modulating class switching in a B cell, comprising introducing an antisense nucleotide sequence into the cell, wherein said antisense nucleotide sequence specifically hybridizes to a nucleic acid 20 molecule encoding a TPBD, wherein said hybridization reduces or inhibits the expression of said TPBD in said cell.

46. A therapeutic composition comprising a compound selected from a TPBD, or functional fragment 25 thereof, a TPBD modulating agent identified according to claim 37, or an anti-TPBD antibody; and a pharmaceutically acceptable carrier.

47. A method of treating a pathology characterized by abnormal cell proliferation or abnormal immunoglobulin class-switching, said method comprising administering an effective amount of the composition  
5 according to claim 46.

48. A method of diagnosing a pathology characterized by an increased or decreased level of a TPBD in a subject, comprising the steps of:

- a) obtaining a test sample from the subject;
- 10 b) contacting said test sample with an agent that can bind said TPBD under suitable conditions, which allow specific binding of said agent to said TPBD; and
- 15 c) comparing the amount of said specific binding in said test sample with the amount of specific binding in a control sample, wherein an increased or decreased amount of said specific binding in said test sample as compared to said control sample is diagnostic  
20 of a pathology.

49. The method of claim 48, wherein said agent is an anti-TPBD antibody, a TNF family receptor, a TRAF protein, or a TRAF-associated protein.

50. A method of modulating the level of  
25 apoptosis in a cell, comprising contacting the cell with an agent that effectively alters the association of TPBD with a TRAF-associated-protein in the cell, or that effectively alters the activity of NF- $\kappa$ B or JNK in the cell.

51. A chimeric protein comprising the TPBD of claim 1.

52. A chimeric TPBD-containing protein, comprising the sequence SEQ ID NO:19, provided said  
5 chimeric protein is not naturally occurring.

53. A chimeric TPBD-containing protein, comprising:

- (a) the sequence SEQ ID NO:19; and
- (b) a sequence from a heterologous protein.

10 54. The chimeric TPBD-containing protein of claim 53, further comprising SEQ ID NO:20.

55. The chimeric TPBD-containing protein of claim 53, further comprising SEQ ID NO:21.

15 56. The chimeric protein of claim 53 further comprising a RING finger domain.

57. An isolated TPBD-containing TRAF protein, or fragment thereof, comprising the sequence SEQ ID NO:19, provided said TRAF domain containing protein does not consist of the sequence SEQ ID NOs:2, 4 or 6.

20 58. The TPBD-containing TRAF protein of claim 57, further comprising the sequence SEQ ID NO:20.

59. The TPBD-containing TRAF protein of claim 57, further comprising the sequence SEQ ID NO:21.

25 60. A TPBD-containing polypeptide comprising the sequence SEQ ID NO:20, provided said polypeptide is no longer than 213 amino acids.

61. The method of claim 37, wherein said agent modulates TPDB association with a TRAF protein, or TPBD association with a TNF family receptor, or TPBD association with a TRAF-associated protein.

5           62. A method of modulating TPBD:TRAF protein interactions comprising contacting a TPBD with the agent of claim 61.

63. The method of claim 37, wherein said agent modulates JNK activity.

10           64. The method of claim 37, wherein said agent modulates NF- $\kappa$ B activity.

15           65. A method of modulating class switching comprising contacting a cell with a compound selected from the group consisting of: a TPBD or functional fragment thereof, an agent identified according to claim 28, and an anti-TPBD antibody.

66. A method of diagnosing cancer or monitoring cancer therapy comprising contacting a test sample from a patient with the antibody of claim 8.

20           67. A method of assessing prognosis of patients with cancer comprising contacting a test sample from a patient with the antibody of claim 8.

68. An effective agent that binds a TRAF protein binding site of TPBD.

25           69. An effective agent that modulates the association of TPBD with a TNF family receptor or a TRAF protein, TRAF protein or a TRAF-associated protein.

70. The agent of claim 69, wherein said TNF family receptor is TNF-R2, said TRAF protein is human TRAF6 and said TRAF-associated protein is I-TRAF.

71. The agent of claim 69, wherein said TNF family receptor is CD40, said TRAF protein is human TRAF2 and said TRAF-associated protein is I-TRAF.

72. The agent of claim 69, wherein said effective agent inhibits the association of said TPBD with said TNF family receptor or a TRAF protein, TRAF protein or a TRAF-associated protein.

73. The agent of claim 69, wherein said effective agent increases the association of said TPBD with said TNF family receptor or a TRAF protein, TRAF protein or a TRAF-associated protein.

**ABSTRACT**

In accordance with the present invention, there are provided novel TRAF-Protein-Binding-Domain polypeptides (TPBDs). The invention also provides  
 5 nucleic acid molecules encoding TPBDs, vectors containing these nucleic acid molecules and host cells containing the vectors. The invention also provides antibodies that can specifically bind to invention TPBDs. Such TPBDs and/or anti-TPBD antibodies are useful for discovery of  
 10 drugs that suppress autoimmunity, inflammation, allergy, allograft rejection, sepsis, and other diseases.

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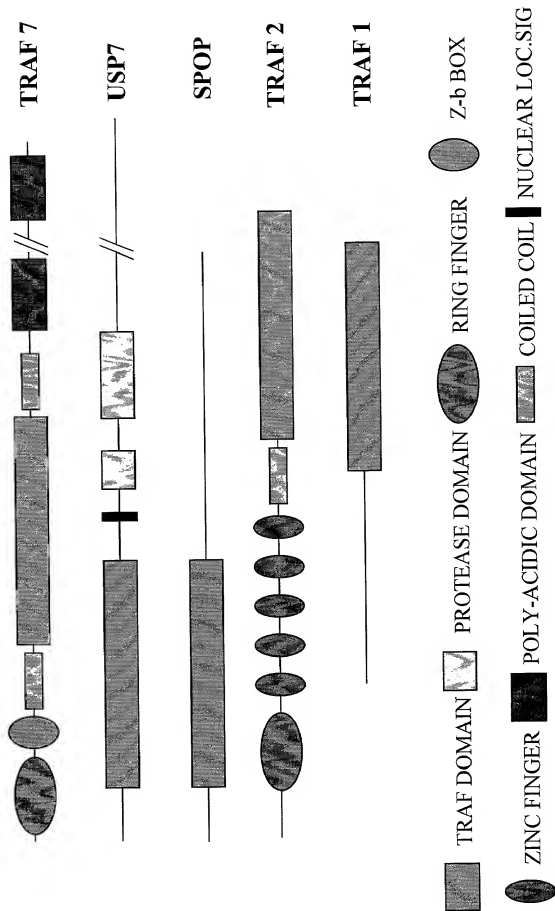


FIGURE 1



FIGURE 2

1 MDEQSVESIAEVFRFCFICMEKLRDARLCPHGSKLCCFSCIRRWLTQRAQQPHCRAPLQL  
 61 RELVNCRWAAEVTQQLDTLQLCSLTKEHENEKDKGCEHHHEKESVFCWITKKKGICHCALW  
 121 GOMHGGHTFKPLAEIYEQHVTKVNEEVAKLRRRLMELISLQCEVERWVEAVRNAKDERVR  
 181 EIRNAVEMMIARLDQLKKNKJITLMGQKTSLTQETELLESLLQVEVHQLRSCSKSELISK  
 241 SSEILMMFQQVHRKPMASVFTTPVPDPFTSELVPSYDSATFVLENFSTLROBAPVWSP  
 301 QVSGLCWRLKVPDNGVVRGYLSVLELSAGILPETSKEYEYRVEVMYHOSNDPTKNII  
 361 REFASDFEVBGECGYNRRFRRLDILANEGYNPQNDTVILRFQVRSPTEFFQKSRDQHWYT  
 421 QLEAAQPSYQQJINLKERTIELSHTQKSRDLSPPDNHLSPPQNDIDALETAKKKSQSDM  
 481 LLEGGPTTASVPEAKEDEDEEKKIQWEDYHHELSQGLDLDLVYEDEVNQLDQSSSSASS  
 541 TATSNTTEENDIDEEITMSGENDVEYNNMELEGEIMEDAAAAAGPAGSSHGCVGSSSRISRR  
 601 THLCSAATSSLLDIDPLILHLLDKDRSSIENLWGLQPRPPASLLQPTASYSRKDKDQR  
 661 KQAMWVRVPSDLKMLKRLKTQMAEVRCMKTDVKNLTSEIKSSAASGDMQTSLSADQAA  
 721 LAACGTENSGRLQDLGMELLAKSSVANCYIRNSTNKKNSPKPARSSVAGSLSLRAVDP  
 781 GENSRKGDQDTLSEGPSGSSQSGSRHSSPRALIHGSGDILPKTEDRQCKALDSDAVV  
 841 AVFSGPLPAVEKRRKMVTLGANAKGQHLEGLQWTDLENNSETGELOPVLPEGASAPEEGM  
 901 SSDSDIEDTENEQEHTSVGGPHDSFVMVMTQPPDETHSSFPDGEQIGPEDLSINTDE  
 961 NSGR

FIGURE 3

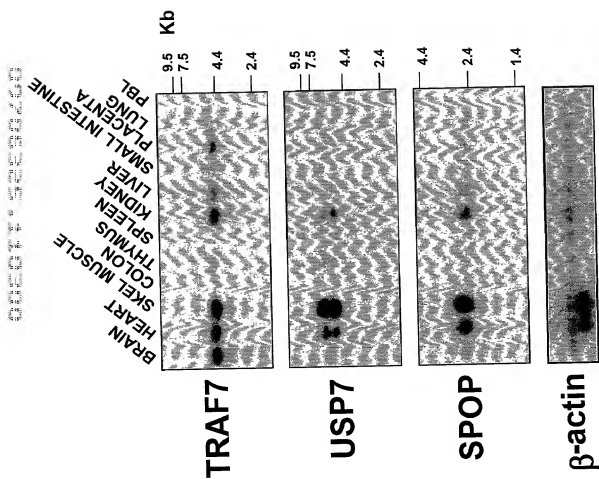


FIGURE 4

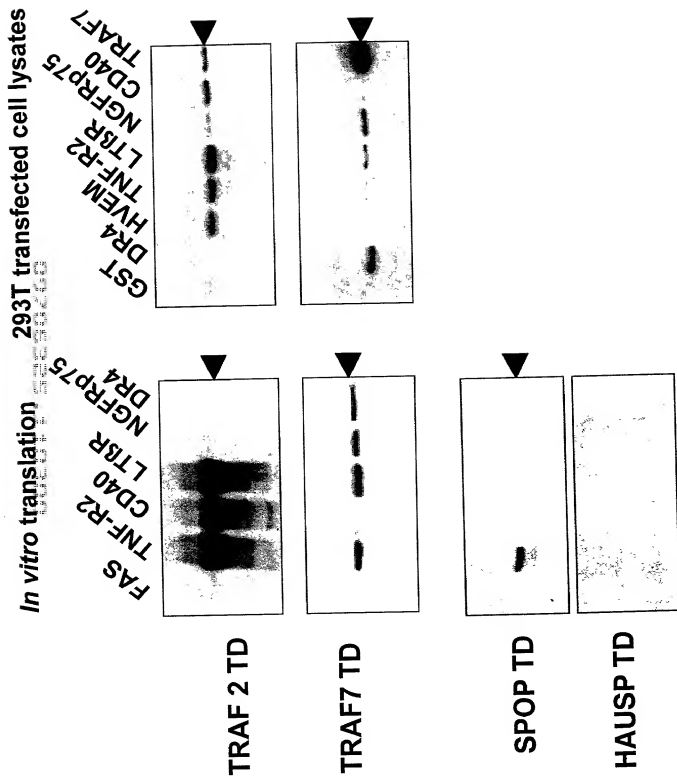


FIGURE 5

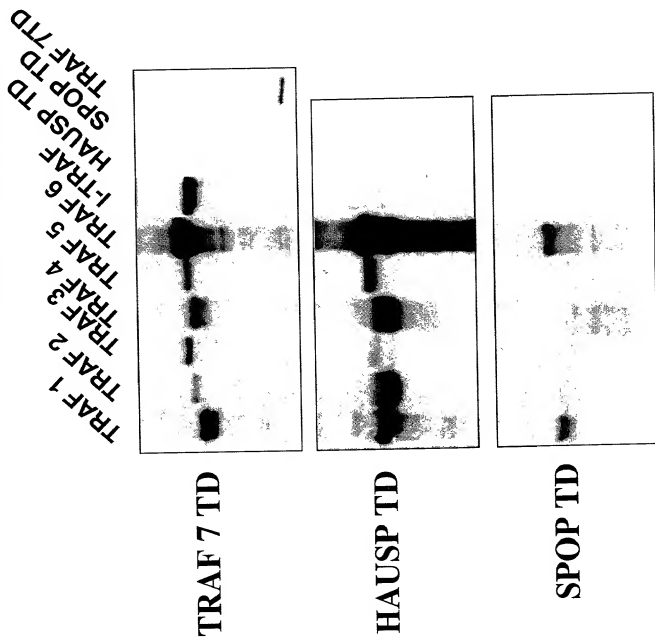


FIGURE 6

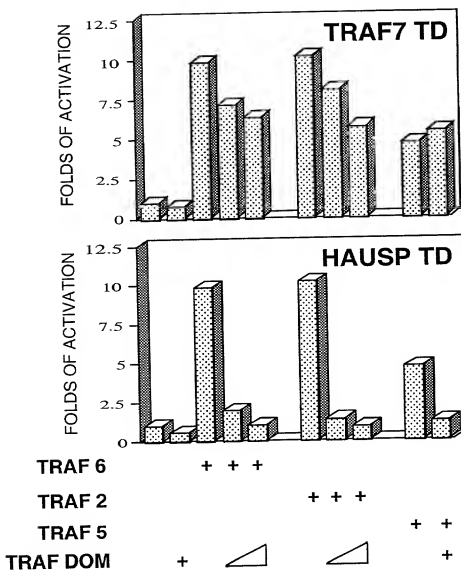


FIGURE 7A

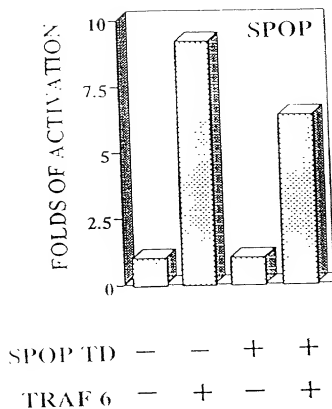


FIGURE 7B

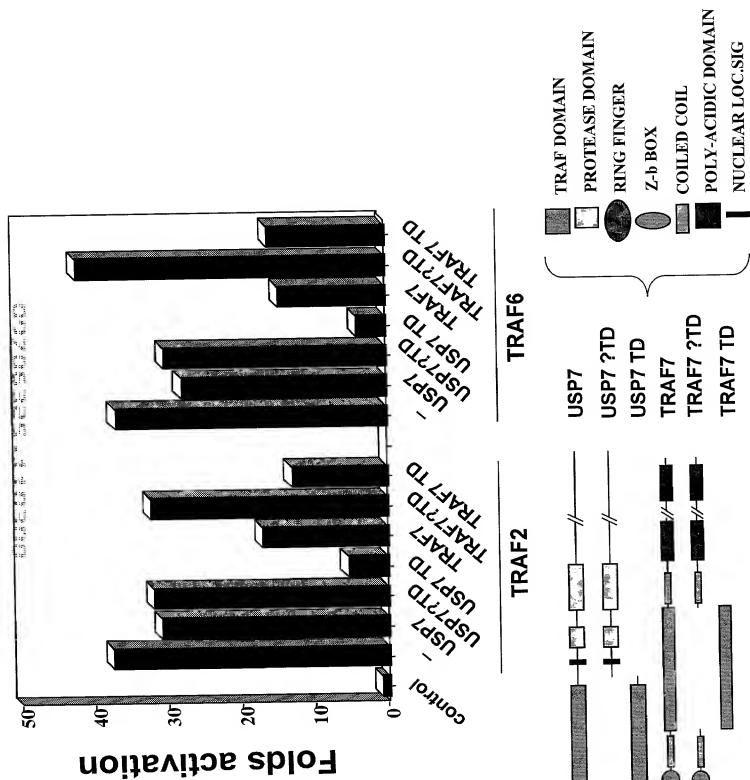


FIGURE 8



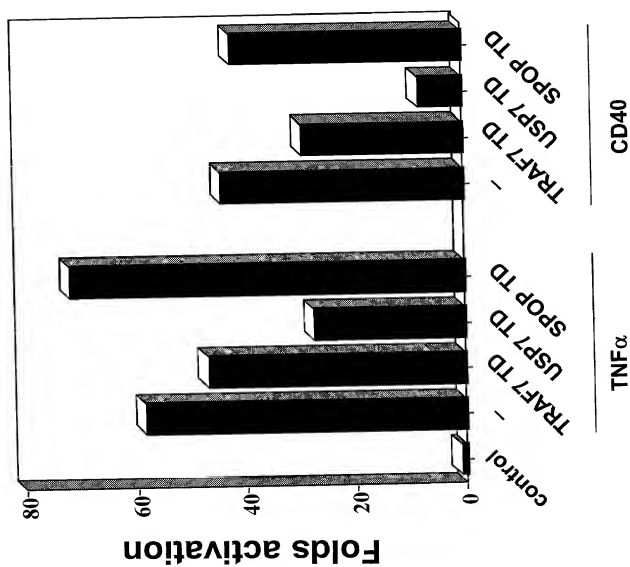


FIGURE 9

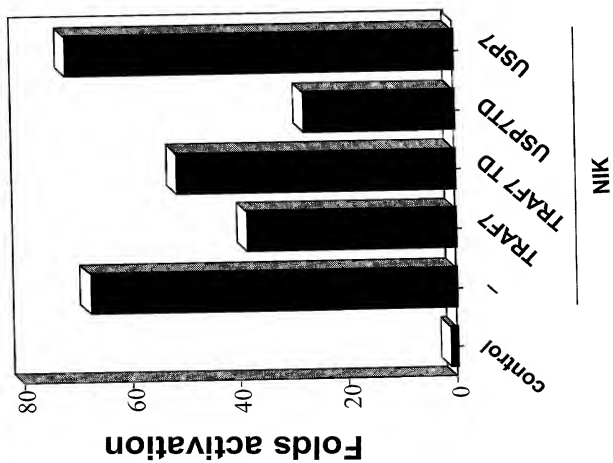


FIGURE 10

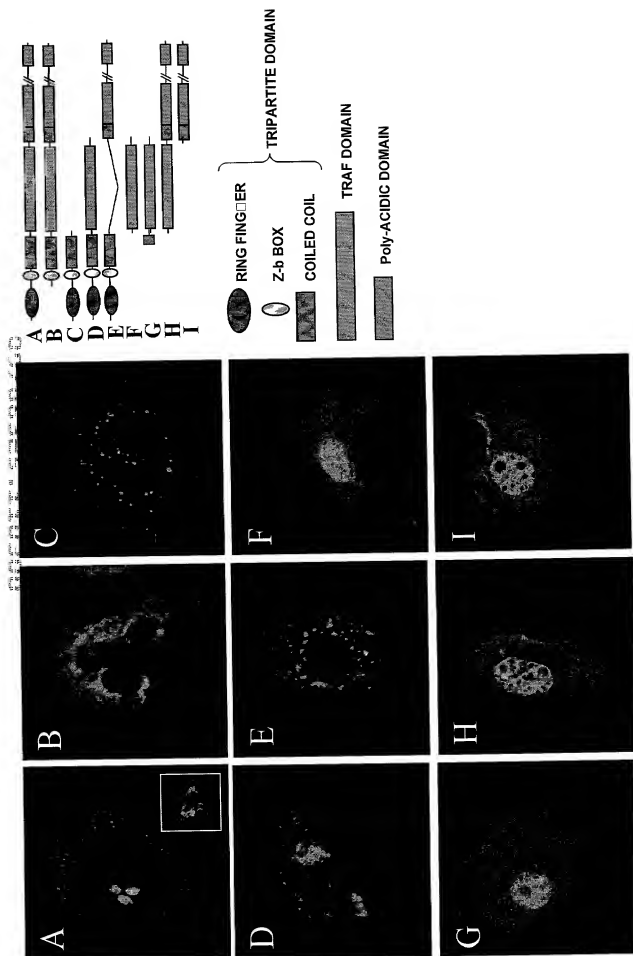


FIGURE 11

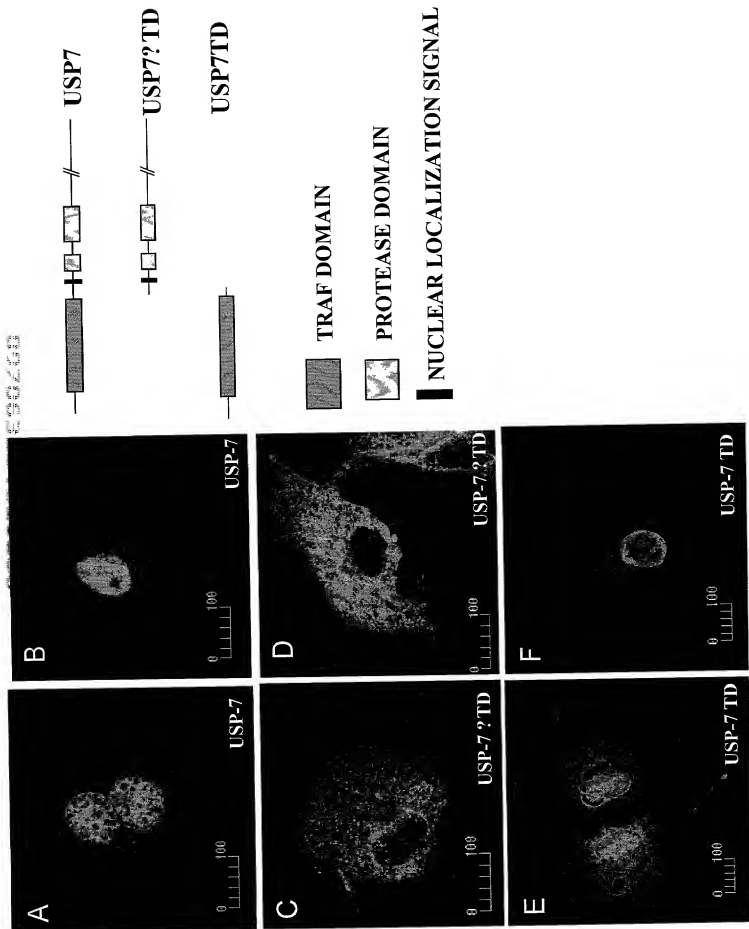
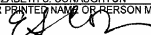


FIGURE 12

PATENT

Our Docket: P-LJ 4453

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Application of	)	CERTIFICATE OF MAILING BY "EXPRESS MAIL"
Zapata and Reed	)	
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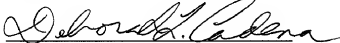
**BOX PATENT APPLICATION**  
Commissioner for Patents  
Washington, D.C. 20231

Sir:

**STATEMENT UNDER 37 C.F.R. § 1.821(f)**

I hereby state that the content of the paper and computer readable copies of the Sequence Listing, submitted in accordance with 37 CFR § 1.821(c) and (e), respectively, are the same.

Respectfully submitted,



Deborah L. Cadena

Registration No. 44,048

Telephone: (858) 535-9001

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November 3, 2000

Date

CAMPBELL & FLORES LLP  
4370 La Jolla Village Drive  
7<sup>th</sup> Floor  
San Diego, California 92122  
USPTO CUSTOMER NO. 23601

2

<110> Reed, John C.  
Zapata, Juan M.

&lt;120&gt; Novel TRAF Family Proteins

&lt;130&gt; P-LJ 4453

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<151> 1999-11-05

&lt;160&gt; 32

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Ala	Trp	Asp	Ser	Lys	Lys	His	Thr	Gly	Tyr	Val	Gly	Leu	Lys	Asn	Gln	
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Glu Leu Ala Ala Ser Gly Ala Thr Leu Pro Lys Phe Asp Lys Asp His	
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Ser Trp Ser Cys His Ala Gln Ala Val Leu Lys Ile Ile Asn Tyr Arg  
130 135 140

Asp Asp Glu Lys Ser Phe Ser Arg Arg Ile Ser His Leu Phe Phe His  
145 150 155 160

Lys Glu Asn Asp Trp Gly Phe Ser Asn Phe Met Ala Trp Ser Glu Val  
165 170 175

Thr Asp Pro Glu Lys Gly Phe Ile Asp Asp Asp Lys Val Thr Phe Glu  
180 185 190

Val Phe Val Gln Ala Asp Ala Pro His Gly Val Ala Trp Asp Ser Lys  
195 200 205

Lys His Thr Gly Tyr Val Gly Leu Lys Asn Gln Gly Ala Thr Cys Tyr  
210 215 220

Met Asn Ser Leu Leu Gln Thr Leu Phe Phe Thr Asn Gln Leu Arg Lys  
225 230 235 240

Ala Val Tyr Met Met Pro Thr Glu Gly Asp Asp Ser Ser Lys Ser Val

245	250	255
Pro Leu Ala Leu Gln Arg Val Phe Tyr Glu Leu Gln His Ser Asp Lys		
260	265	270
Pro Val Gly Thr Lys Lys Leu Thr Lys Ser Phe Gly Trp Glu Thr Leu		
275	280	285
Asp Ser Phe Met Gln His Asp Val Gln Glu Leu Cys Arg Val Leu Leu		
290	295	300
Asp Asn Val Glu Asn Lys Met Lys Gly Thr Cys Val Glu Gly Thr Ile		
305	310	315
Pro Lys Leu Phe Arg Gly Lys Met Val Ser Tyr Ile Gln Cys Lys Glu		
325	330	335
Val Asp Tyr Arg Ser Asp Arg Arg Glu Asp Tyr Tyr Asp Ile Gln Leu		
340	345	350
Ser Ile Lys Gly Lys Lys Asn Ile Phe Glu Ser Phe Val Asp Tyr Val		
355	360	365
Ala Val Glu Gln Leu Asp Gly Asp Asn Lys Tyr Asp Ala Gly Glu His		
370	375	380
Gly Leu Gln Glu Ala Glu Lys Gly Val Lys Phe Leu Thr Leu Pro Pro		
385	390	395
Val Leu His Leu Gln Leu Met Arg Phe Met Tyr Asp Pro Gln Thr Asp		
405	410	415
Gln Asn Ile Lys Ile Asn Asp Arg Phe Glu Phe Pro Glu Gln Leu Pro		
420	425	430
Leu Asp Glu Phe Leu Gln Lys Thr Asp Pro Lys Asp Pro Ala Asn Tyr		
435	440	445
Ile Leu His Ala Val Leu Val His Ser Gly Asp Asn His Gly Gly His		
450	455	460
Tyr Val Val Tyr Leu Asn Pro Lys Gly Asp Gly Lys Trp Cys Lys Phe		
465	470	475
Asp Asp Asp Val Val Ser Arg Cys Thr Lys Glu Glu Ala Ile Glu His		
485	490	495
Asn Tyr Gly Gly His Asp Asp Asp Leu Ser Val Arg His Cys Thr Asn		

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Ala Tyr Met Leu Val Tyr Ile Arg Glu Ser Lys Leu Ser Glu Val Leu		
515	520	525
Gln Ala Val Thr Asp His Asp Ile Pro Gln Gln Leu Val Glu Arg Leu		
530	535	540
Gln Glu Glu Lys Arg Ile Glu Ala Gln Lys Arg Lys Glu Arg Gln Glu		
545	550	555
Ala His Leu Tyr Met Gln Val Gln Ile Val Ala Glu Asp Gln Phe Cys		
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Gly His Gln Gly Asn Asp Met Tyr Asp Glu Glu Lys Val Lys Tyr Thr		
580	585	590
Val Phe Lys Val Leu Lys Asn Ser Ser Leu Ala Glu Phe Val Gln Ser		
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Leu Ser Gln Thr Met Gly Phe Pro Gln Asp Gln Ile Arg Leu Trp Pro		
610	615	620
Met Gln Ala Arg Ser Asn Gly Thr Lys Arg Pro Ala Met Leu Asp Asn		
625	630	635
Glu Ala Asp Gly Asn Lys Thr Met Ile Glu Leu Ser Asp Asn Glu Asn		
645	650	655
Pro Trp Thr Ile Phe Leu Glu Thr Val Asp Pro Glu Leu Ala Ala Ser		
660	665	670
Gly Ala Thr Leu Pro Lys Phe Asp Lys Asp His Asp Val Met Leu Phe		
675	680	685
Leu Lys Met Tyr Asp Pro Lys Thr Arg Ser Leu Asn Tyr Cys Gly His		
690	695	700
Ile Tyr Thr Pro Ile Ser Cys Lys Ile Arg Asp Leu Leu Pro Val Met		
705	710	715
Cys Asp Arg Ala Gly Phe Ile Gln Asp Thr Ser Leu Ile Leu Tyr Glu		
725	730	735
Glu Val Lys Pro Asn Leu Thr Glu Arg Ile Gln Asp Tyr Asp Val Ser		
740	745	750
Leu Asp Lys Ala Leu Asp Glu Leu Met Asp Gly Asp Ile Ile Val Phe		

755

760

765

Gln Lys Asp Asp Pro Glu Asn Asp Asn Ser Glu Leu Pro Thr Ala Lys  
770 775 780

Glu Tyr Phe Arg Asp Leu Tyr His Arg Val Asp Val Ile Phe Cys Asp  
785 790 795 800

Lys Thr Ile Pro Asn Asp Pro Gly Phe Val Val Thr Leu Ser Asn Arg  
805 810 815

Met Asn Tyr Phe Gln Val Ala Lys Thr Val Ala Gln Arg Leu Asn Thr  
820 825 830

Asp Pro Met Leu Leu Gln Phe Phe Lys Ser Gln Gly Tyr Arg Asp Gly  
835 840 845

Pro Gly Asn Pro Leu Arg His Asn Tyr Glu Gly Thr Leu Arg Asp Leu  
850 855 860

Leu Gln Phe Phe Lys Pro Arg Gln Pro Lys Lys Leu Tyr Tyr Gln Gln  
865 870 875 880

Leu Lys Met Lys Ile Thr Asp Phe Glu Asn Arg Arg Ser Phe Lys Cys  
885 890 895

Ile Trp Leu Asn Ser Gln Phe Arg Glu Glu Glu Ile Thr Leu Tyr Pro  
900 905 910

Asp Lys His Gly Cys Val Arg Asp Leu Leu Glu Glu Cys Lys Lys Ala  
915 920 925

Val Glu Leu Gly Glu Lys Ala Ser Gly Lys Leu Arg Leu Leu Glu Ile  
930 935 940

Val Ser Tyr Lys Lys Ile Ile Gly Val His Gln Glu Asp Glu Leu Leu Glu  
945 950 955 960

Cys Leu Ser Pro Ala Thr Ser Arg Thr Phe Arg Ile Glu Glu Ile Pro  
965 970 975

Leu Asp Gln Val Asp Ile Asp Lys Glu Asn Glu Met Leu Val Thr Val  
980 985 990

Ala His Phe His Lys Glu Val Phe Gly Thr Phe Gly Ile Pro Phe Leu  
995 1000 1005

Leu Arg Ile His Gln Gly Glu His Phe Arg Glu Val Met Lys Arg Ile



1010

1015

1020

Gln Ser Leu Leu Asp Ile Gln Glu Lys Glu Phe Glu Lys Phe Lys Phe  
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Ala Ile Val Met Thr Gly Arg His Gln Tyr Ile Asn Glu Asp Glu Tyr  
 1045 1050 1055

Glu Val Asn Leu Lys Asp Phe Glu Pro Gln Pro Gly Asn Met Ser His  
 1060 1065 1070

Pro Arg Pro Trp Leu Gly Leu Asp His Phe Asn Lys Ala Pro Lys Arg  
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Ser Arg Tyr Thr Tyr Leu Glu Lys Ala Ile Lys Ile His Asn  
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 ctgacttttg aaatctcggt taaccttcaa actggcg atg tca agg gtt cca agt 175  
 Met Ser Arg Val Pro Ser  
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cct cca cct ccg gca gaa atg tcg agt ggc ccc gta gct gag agt tgg 223  
 Pro Pro Pro Pro Ala Glu Met Ser Ser Gly Pro Val Ala Glu Ser Trp  
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tgc tac aca cag atc aag gta gtg aaa ttc tcc tac atg tgg acc atc 271  
 Cys Tyr Thr Gln Ile Lys Val Val Lys Phe Ser Tyr Met Trp Thr Ile  
 25 30 35

aat aac ttt agc ttt tgc cgg gag gaa atg ggt gaa gtc att aaa agt 319  
 Asn Asn Phe Ser Phe Cys Arg Glu Glu Met Gly Glu Val Ile Lys Ser  
 40 45 50

tct aca ttt tca tca gga gca aat gat aaa ctg aaa tgg tgt ttg cga	367
Ser Thr Phe Ser Ser Gly Ala Asn Asp Lys Leu Lys Trp Cys Leu Arg	
55 60 65 70	
gta aac ccc aaa ggg tta gat gaa gaa agc aaa gat tac ctg tca ctt	415
Val Asn Pro Lys Gly Leu Asp Glu Glu Ser Lys Asp Tyr Leu Ser Leu	
75 80 85	
tac ctg tta ctg gtc agc tgt cca aag agt gaa gtt cgg gca aaa ttc	463
Tyr Leu Leu Leu Val Ser Cys Pro Lys Ser Glu Val Arg Ala Lys Phe	
90 95 100	
aaa ttc tcc atc ctg aat gcc aag gga gaa gaa acc aaa gct atg gag	511
Lys Phe Ser Ile Leu Asn Ala Lys Gly Glu Glu Thr Lys Ala Met Glu	
105 110 115	
agt caa cgg gca tat agg ttt gtg caa ggc aaa gac tgg gga ttc aag	559
Ser Gln Arg Ala Tyr Arg Phe Val Gln Gly Lys Asp Trp Gly Phe Lys	
120 125 130	
aaa ttc atc cgt aga gat ttt ctt ttg gat gag gcc aac ggg ctt ctc	607
Lys Phe Ile Arg Arg Asp Phe Leu Leu Asp Glu Ala Asn Gly Leu Leu	
135 140 145 150	
cct gat gac aag ctt acc ctc ttc tgc gag gtg agt gtt gtg caa gat	655
Pro Asp Asp Lys Leu Thr Leu Phe Cys Glu Val Ser Val Val Gln Asp	
155 160 165	
tct gtc aac att tct ggc cag aat acc atg aac atg gta aag gtt cct	703
Ser Val Asn Ile Ser Gly Gln Asn Thr Met Asn Met Val Lys Val Pro	
170 175 180	
gag tgc cgg ctg gca gat gag tta gga gga ctg tgg gag aat tcc cgg	751
Glu Cys Arg Leu Ala Asp Glu Leu Gly Gly Leu Trp Glu Asn Ser Arg	
185 190 195	
ttc aca gac tgc tgc ttg tgt gtt gcc ggc cag gaa ttc cag gct cac	799
Phe Thr Asp Cys Cys Leu Cys Val Ala Gly Gln Glu Phe Gln Ala His	
200 205 210	
aag gct atc tta gca gct cgt tct ccg gtt ttt agt gcc atg ttt gaa	847
Lys Ala Ile Leu Ala Ala Arg Ser Pro Val Phe Ser Ala Met Phe Glu	
215 220 225 230	
cat gaa atg gag gag agc aaa aag aat cga gtt gaa atc aat gat gtg	895
His Glu Met Glu Glu Ser Lys Lys Asn Arg Val Glu Ile Asn Asp Val	
235 240 245	

gag cct gaa gtt ttt aag gaa atg atg tgc ttc att tac acg ggg aag 943  
 Glu Pro Glu Val Phe Lys Glu Met Met Cys Phe Ile Tyr Thr Gly Lys  
 250 255 260

gct cca aac ctc gac aaa atg gct gat gat ttg ctg gca gct gct gac 991  
 Ala Pro Asn Leu Asp Lys Met Ala Asp Asp Leu Leu Ala Ala Ala Asp  
 265 270 275

aag tat gcc ctg gag cgc tta aag gtc atg tgt gag gat gcc ctc tgc 1039  
 Lys Tyr Ala Leu Glu Arg Leu Lys Val Met Cys Glu Asp Ala Leu Cys  
 280 285 290

agt aac ctg tcc gtg gag aac gct gca gaa att ctc atc ctg gcc gac 1087  
 Ser Asn Leu Ser Val Glu Asn Ala Ala Glu Ile Leu Ile Leu Ala Asp  
 295 300 305 310

ctc cac agt gca gat cag ttg aaa act cag gca gtg gat ttc atc aac 1135  
 Leu His Ser Ala Asp Gln Leu Lys Thr Gln Ala Val Asp Phe Ile Asn  
 315 320 325

tat cat gct tcg gat gtc ttg gag acc tct ggg tgg aag tca atg gtg 1183  
 Tyr His Ala Ser Asp Val Leu Glu Thr Ser Gly Trp Lys Ser Met Val  
 330 335 340

gtg tca cat ccc cac ttg gtg gct gag gca tac cgc tct ctg gct tca 1231  
 Val Ser His Pro His Leu Val Ala Glu Ala Tyr Arg Ser Leu Ala Ser  
 345 350 355

gca cag tgc cct ttt ctg gga ccc cca cgc aaa cgc ctg aag caa tcc 1279  
 Ala Gln Cys Pro Phe Leu Gly Pro Pro Arg Lys Arg Leu Lys Gln Ser  
 360 365 370

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 ccaactgacca ccaggtagac agcgcaatct gtggagcttt tactctgttg tgagggaag 1399  
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 gtttgatact gtggggatlc agtttaggcg ctggcccag gatatccag cggtgggtact 1579  
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35 40 45

Gly Glu Val Ile Lys Ser Ser Thr Phe Ser Ser Gly Ala Asn Asp Lys  
50 55 60

Leu Lys Trp Cys Leu Arg Val Asn Pro Lys Gly Leu Asp Glu Glu Ser  
65 70 75 80

Lys Asp Tyr Leu Ser Leu Tyr Leu Leu Leu Val Ser Cys Pro Lys Ser  
85 90 95

Glu Val Arg Ala Lys Phe Lys Phe Ser Ile Leu Asn Ala Lys Gly Glu  
100 105 110

Glu Thr Lys Ala Met Glu Ser Gln Arg Ala Tyr Arg Phe Val Gln Gly  
115 120 125

Lys Asp Trp Gly Phe Lys Lys Phe Ile Arg Arg Asp Phe Leu Leu Asp  
130 135 140

Glu Ala Asn Gly Leu Leu Pro Asp Asp Lys Leu Thr Leu Phe Cys Glu  
145 150 155 160

Val Ser Val Val Gln Asp Ser Val Asn Ile Ser Gly Gln Asn Thr Met  
165 170 175

Asn Met Val Lys Val Pro Glu Cys Arg Leu Ala Asp Glu Leu Gly Gly  
180 185 190

Leu Trp Glu Asn Ser Arg Phe Thr Asp Cys Cys Leu Cys Val Ala Gly  
195 200 205

Gln Glu Phe Gln Ala His Lys Ala Ile Leu Ala Ala Arg Ser Pro Val  
210 215 220

Phe Ser Ala Met Phe Glu His Glu Met Glu Glu Ser Lys Lys Asn Arg

225                      230                      235                      240

Val Glu Ile Asn Asp Val Glu Pro Glu Val Phe Lys Glu Met Met Cys  
                                  245                      250                      255

Phe Ile Tyr Thr Gly Lys Ala Pro Asn Leu Asp Lys Met Ala Asp Asp  
                                  260                      265                      270

Leu Leu Ala Ala Ala Asp Lys Tyr Ala Leu Glu Arg Leu Lys Val Met  
                                  275                      280                      285

Cys Glu Asp Ala Leu Cys Ser Asn Leu Ser Val Glu Asn Ala Ala Glu  
                                  290                      295                      300

Ile Leu Ile Leu Ala Asp Leu His Ser Ala Asp Gln Leu Lys Thr Gln  
 305                      310                      315                      320

Ala Val Asp Phe Ile Asn Tyr His Ala Ser Asp Val Leu Glu Thr Ser  
                                  325                      330                      335

Gly Trp Lys Ser Met Val Val Ser His Pro His Leu Val Ala Glu Ala  
                                  340                      345                      350

Tyr Arg Ser Leu Ala Ser Ala Gln Cys Pro Phe Leu Gly Pro Pro Arg  
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Lys Arg Leu Lys Gln Ser  
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gat gaa cag agc gtg gag agc att gct gag gtt ttc cga tgt ttc att    96  
 Asp Glu Gln Ser Val Glu Ser Ile Ala Glu Val Phe Arg Cys Phe Ile  
                                  20                      25                      30

tgt atg gag aaa ttg cgg gat gca cgc ctg tgt cct cat tgc tcc aaa	144
Cys Met Glu Lys Leu Arg Asp Ala Arg Leu Cys Pro His Cys Ser Lys	
35 40 45	
ctg tgt tgt ttc agc tgt att agg cgc tgg ctg aca gag cag aga gct	192
Leu Cys Cys Phe Ser Cys Ile Arg Arg Trp Leu Thr Glu Gln Arg Ala	
50 55 60	
caa tgt cct cat tgc cgt gct cca ctc cag cta cga gaa cta gta aat	240
Gln Cys Pro His Cys Arg Ala Pro Leu Gln Leu Arg Glu Leu Val Asn	
65 70 75 80	
tgt cgt tgg gca gaa gaa gta aca caa cag ctt gat act ctt caa ctc	288
Cys Arg Trp Ala Glu Glu Val Thr Gln Gln Leu Asp Thr Leu Gln Leu	
85 90 95	
tgc agt ctc acc aaa cat gaa gaa aat gaa aag gac aaa tgt gaa aat	336
Cys Ser Leu Thr Lys His Glu Glu Asn Glu Lys Asp Lys Cys Glu Asn	
100 105 110	
cac cat gaa aaa ctt agt gta ttt tgc tgg act tgt aag aag tgt atc	384
His His Glu Lys Leu Ser Val Phe Cys Trp Thr Cys Lys Lys Cys Ile	
115 120 125	
tgc cat cag tgt gca ctt tgg gga gga atg cat ggc gga cat acc ttt	432
Cys His Gln Cys Ala Leu Trp Gly Gly Met His Gly Gly His Thr Phe	
130 135 140	
aaa cct ttg gca gaa att tat gag caa cac gtc act aaa gtg aat gaa	480
Lys Pro Leu Ala Glu Ile Tyr Glu Gln His Val Thr Lys Val Asn Glu	
145 150 155 160	
gag gta gcc aaa ctt cgt cgg cgt ctc atg gaa ctg atc agc tta gtt	528
Glu Val Ala Lys Leu Arg Arg Arg Leu Met Glu Leu Ile Ser Leu Val	
165 170 175	
caa gaa gtg gaa agg aat gta gaa gct gta aga aat gca aaa gat gag	576
Gln Glu Val Glu Arg Asn Val Glu Ala Val Arg Asn Ala Lys Asp Glu	
180 185 190	
cgt gtt cgg gaa att agg aat gca gtg gag atg atg att gca cgg tta	624
Arg Val Arg Glu Ile Arg Asn Ala Val Glu Met Met Ile Ala Arg Leu	
195 200 205	
gac aca cag ctg aag aat aag ctt ata aca ctg atg ggt cag aag aca	672
Asp Thr Gln Leu Lys Asn Lys Leu Ile Thr Leu Met Gly Gln Lys Thr	
210 215 220	

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Ser	Leu	Thr	Gln	Glu	Thr	Glu	Leu	Leu	Glu	Ser	Leu	Leu	Gln	Glu	Val	
225					230					235					240	
gag	cac	cag	ttg	cgg	tct	tgt	agt	aag	agt	gag	ttg	ata	tct	aag	agc	768
Glu	His	Gln	Leu	Arg	Ser	Cys	Ser	Lys	Ser	Glu	Leu	Ile	Ser	Lys	Ser	
				245					250						255	
tca	gag	atc	ctt	atg	atg	ttt	cag	caa	gtt	cat	cgg	aag	ccc	atg	gca	816
Ser	Glu	Ile	Leu	Met	Met	Phe	Gln	Gln	Val	His	Arg	Lys	Pro	Met	Ala	
			260					265					270			
tct	ttt	gtt	acc	act	cct	gtt	cca	cca	gac	ttt	acc	agt	gaa	tta	gtg	864
Ser	Phe	Val	Thr	Thr	Pro	Val	Pro	Pro	Asp	Phe	Thr	Ser	Glu	Leu	Val	
		275					280					285				
cca	tct	tac	gat	tca	gct	act	ttt	gtt	tta	gag	aat	tto	agc	act	ttg	912
Pro	Ser	Tyr	Asp	Ser	Ala	Thr	Phe	Val	Leu	Glu	Asn	Phe	Ser	Thr	Leu	
		290				295					300					
cgt	cag	aga	gca	gat	cct	gtt	tac	agt	cca	cct	ctt	caa	gtt	tca	gga	960
Arg	Gln	Arg	Ala	Asp	Pro	Val	Tyr	Ser	Pro	Pro	Leu	Gln	Val	Ser	Gly	
305					310					315					320	
ctt	tcg	tggt	agg	tta	aaa	gtt	tac	cca	gat	gga	aat	gga	gtt	gtg	cga	1008
Leu	Cys	Trp	Arg	Leu	Lys	Val	Tyr	Pro	Asp	Gly	Asn	Gly	Val	Val	Arg	
				325					330					335		
ggt	tac	tac	tta	tct	gtg	ttt	ctg	gag	ctc	tca	gct	ggc	ttg	cct	gaa	1056
Gly	Tyr	Tyr	Leu	Ser	Val	Phe	Leu	Glu	Leu	Ser	Ala	Gly	Leu	Pro	Glu	
			340					345					350			
act	tct	aaa	tat	gaa	tat	cgt	gta	gag	atg	gtt	cac	cag	tcc	tgt	aat	1104
Thr	Ser	Lys	Tyr	Glu	Tyr	Arg	Val	Glu	Met	Val	His	Gln	Ser	Cys	Asn	
		355					360					365				
gat	cct	aca	aaa	aat	atc	att	cga	gaa	ttt	gca	tct	gac	ttt	gaa	gtt	1152
Asp	Pro	Thr	Lys	Asn	Ile	Ile	Arg	Glu	Phe	Ala	Ser	Asp	Phe	Glu	Val	
		370				375					380					
gga	gaa	tcg	tggt	ggc	tat	aat	aga	ttt	ttc	cgt	ttg	gac	tta	ctc	gca	1200
Gly	Glu	Cys	Trp	Gly	Tyr	Asn	Arg	Phe	Phe	Arg	Leu	Asp	Leu	Leu	Ala	
385					390					395					400	
aat	gaa	gga	tac	ttg	aat	cca	caa	aat	gat	aca	gtg	att	tta	agg	ttt	1248
Asn	Glu	Gly	Tyr	Leu	Asn	Pro	Gln	Asn	Asp	Thr	Val	Ile	Leu	Arg	Phe	
				405				410						415		

cag gta cgt tca cca act ttc ttt caa aaa tcc cgg gac cag cat tgg	1296
Gln Val Arg Ser Pro Thr Phe Phe Gln Lys Ser Arg Asp Gln His Trp	
420 425 430	
tac att act cag ttg gaa gct gca cag act agt tat atc caa caa ata	1344
Tyr Ile Thr Gln Leu Glu Ala Ala Gln Thr Ser Tyr Ile Gln Gln Ile	
435 440 445	
aac aac ctt aaa gag aga ctt act att gag ctg tct cga act cag aag	1392
Asn Asn Leu Lys Glu Arg Leu Thr Ile Glu Leu Ser Arg Thr Gln Lys	
450 455 460	
tca aga gat ttg tca cca cca gat aac cat ctt agc ccc caa aat gat	1440
Ser Arg Asp Leu Ser Pro Pro Asp Asn His Leu Ser Pro Gln Asn Asp	
465 470 475 480	
gat gct ctg gag aca cga gct aag aag tct gca tgc tct gac atg ctt	1488
Asp Ala Leu Glu Thr Arg Ala Lys Lys Ser Ala Cys Ser Asp Met Leu	
485 490 495	
ctc gaa ggt ggt cct act aca gct tct gta aga gag gcc aaa gag gat	1536
Leu Glu Gly Gly Pro Thr Thr Ala Ser Val Arg Glu Ala Lys Glu Asp	
500 505 510	
gaa gaa gat gag gag aag att cag aat gaa gat tat cat cac gag ctt	1584
Glu Glu Asp Glu Glu Lys Ile Gln Asn Glu Asp Tyr His His Glu Leu	
515 520 525	
tca gat gga gat ctg gat ctg gat ctt gtt tat gag gat gaa gta aat	1632
Ser Asp Gly Asp Leu Asp Leu Asp Leu Val Tyr Glu Asp Glu Val Asn	
530 535 540	
cag ctc gat ggc agc agt tcc tct gct agt tcc aca gca aca agt aat	1680
Gln Leu Asp Gly Ser Ser Ser Ala Ser Ser Thr Ala Thr Ser Asn	
545 550 555 560	
aca gaa gaa aat gat att gat gaa gaa act atg tct gga gaa aat gat	1728
Thr Glu Glu Asn Asp Ile Asp Glu Glu Thr Met Ser Gly Glu Asn Asp	
565 570 575	
gtg gaa tat aac aac atg gaa tta gaa gag gga gaa ctc atg gaa gat	1776
Val Glu Tyr Asn Asn Met Glu Leu Glu Glu Gly Glu Leu Met Glu Asp	
580 585 590	
gca gct gct gca gga ccc gca ggt agt agc cat ggt tat gtg ggt tcc	1824
Ala Ala Ala Ala Gly Pro Ala Gly Ser Ser His Gly Tyr Val Gly Ser	
595 600 605	



agt agt aga ata tca aga aga aca cat tta tgc tcc gct gct acc agt 1872  
 Ser Ser Arg Ile Ser Arg Arg Thr His Leu Cys Ser Ala Ala Thr Ser  
 610 615 620

agt tta cta gac att gat cca tta att tta ata cat ttg ttg gac ctt 1920  
 Ser Leu Leu Asp Ile Asp Pro Leu Ile Leu Ile His Leu Leu Asp Leu  
 625 630 635 640

aag gac cgg agc agt ata gaa aat ttg tgg ggc tta cag cct cgc cca 1968  
 Lys Asp Arg Ser Ser Ile Glu Asn Leu Trp Gly Leu Gln Pro Arg Pro  
 645 650 655

cct gct tca ctt ctg cag ccc aca gca tca tat tct cga aaa gat aaa 2016  
 Pro Ala Ser Leu Leu Gln Pro Thr Ala Ser Tyr Ser Arg Lys Asp Lys  
 660 665 670

gac caa agg aag caa cag gca atg tgg cga gtg ccc tct gat tta aag 2064  
 Asp Gln Arg Lys Gln Gln Ala Met Trp Arg Val Pro Ser Asp Leu Lys  
 675 680 685

atg cta aaa aga ctc aaa act caa atg gcc gaa gtt cga tgt atg aaa 2112  
 Met Leu Lys Arg Leu Lys Thr Gln Met Ala Glu Val Arg Cys Met Lys  
 690 695 700

act gat gta aag aat aca ctt tca gaa ata aaa agc agc agt gct gct 2160  
 Thr Asp Val Lys Asn Thr Leu Ser Glu Ile Lys Ser Ser Ser Ala Ala  
 705 710 715 720

tct gga gac atg cag aca agc ctt ttt tct gct gac cag gca gct ctg 2208  
 Ser Gly Asp Met Gln Thr Ser Leu Phe Ser Ala Asp Gln Ala Ala Leu  
 725 730 735

gct gca tgt gga act gaa aac tct gcc aga ttg cag gat ttg gga atg 2256  
 Ala Ala Cys Gly Thr Glu Asn Ser Gly Arg Leu Gln Asp Leu Gly Met  
 740 745 750

gaa ctc ctg gca aag tca tca gtt gcc aat tgt tac ata cga aac tcc 2304  
 Glu Leu Leu Ala Lys Ser Ser Val Ala Asn Cys Tyr Ile Arg Asn Ser  
 755 760 765

aca aat aag aag agt aat tcg ccc aag cca gct cga tcc agt gta gca 2352  
 Thr Asn Lys Lys Ser Asn Ser Pro Lys Pro Ala Arg Ser Ser Val Ala  
 770 775 780

ggt agt cta tca ctt cga aga gca gtg gac cct gga gaa aat agt cgt 2400  
 Gly Ser Leu Ser Leu Arg Arg Ala Val Asp Pro Gly Glu Asn Ser Arg  
 785 790 795 800

tca aag gga gac tgt cag act ctg tct gaa ggc tcc cca gga agc tct	2448
Ser Lys Gly Asp Cys Gln Thr Leu Ser Glu Gly Ser Pro Gly Ser Ser	
805 810 815	
cag tct ggg agc agg cac agt tct ccc cga gcc ttg ata cat ggc agt	2496
Gln Ser Gly Ser Arg His Ser Ser Pro Arg Ala Leu Ile His Gly Ser	
820 825 830	
atc ggt gat att ctg cca aaa act gaa gac cgg cag tgt aaa gct ttg	2544
Ile Gly Asp Ile Leu Pro Lys Thr Glu Asp Arg Gln Cys Lys Ala Leu	
835 840 845	
gat tca gat gct gtt gtg gtt gca gtt ttc agt ggc ttg cct gcg gtt	2592
Asp Ser Asp Ala Val Val Ala Val Phe Ser Gly Leu Pro Ala Val	
850 855 860	
gag aaa agg agg aaa atg gtc acc ttg ggg gct aat gct aaa gga ggt	2640
Glu Lys Arg Arg Lys Met Val Thr Leu Gly Ala Asn Ala Lys Gly Gly	
865 870 875 880	
cat ctg gaa gga ctg cag atg act gat ttg gaa aat aat tct gaa act	2688
His Leu Glu Gly Leu Gln Met Thr Asp Leu Glu Asn Asn Ser Glu Thr	
885 890 895	
gga gag tta cag cct gta cta cct gaa gga gct tca gct gcc cct gaa	2736
Gly Glu Leu Gln Pro Val Leu Pro Glu Gly Ala Ser Ala Ala Pro Glu	
900 905 910	
gaa gga atg agt agc gac agt gac att gaa tgt gac act gag aat gag	2784
Glu Gly Met Ser Ser Asp Ser Asp Ile Glu Cys Asp Thr Glu Asn Glu	
915 920 925	
gag cag gaa gag cat acc agt gtg ggc ggg ttt cac gac tcc ttc atg	2832
Glu Gln Glu Glu His Thr Ser Val Gly Gly Phe His Asp Ser Phe Met	
930 935 940	
gtc atg aca cag ccc cag gat gaa gat aca cat tcc agt ttt cct gat	2880
Val Met Thr Gln Pro Pro Asp Glu Asp Thr His Ser Ser Phe Pro Asp	
945 950 955 960	
ggt gaa caa ata ggc cct gaa gat ctc agc ttc aat aca gat gaa aat	2928
Gly Glu Gln Ile Gly Pro Glu Asp Leu Ser Phe Asn Thr Asp Glu Asn	
965 970 975	
agt gga agg taattgccaa atcaagagaa ctgacttgca agctaccttg	2977
Ser Gly Arg	
acctgaatt ttgctgtagt tgggtgctcaa atttgtcatc agtcagataa tcagatttgg	3037

tcttatttct tcattatctc gaactgaaat agtaatttgg aaactgttgg aaggtggcac 3097  
 agtttagtct aagacagcag tagtacatgg gaaaaacagt atgggaagag ttctttgtaa 3157  
 tgtaaggaaa taacaatgta gttctctatt aatttagcaa atttgtacat tcacaaaagg 3217  
 cagtttgtct actacagcag aaggctgggt aactgccaga aaatgtacct ccaggccctg 3277  
 catgccgtca gtaaccgcc cggcattggt gctctactgt ctttggctag agcttagttg 3337  
 tgtttaaata atcatcttta tttttgggtt ttaattaca gttccattag tgccgtgaga 3397  
 ttagtgaaca gaaaattgct ttggaagaga ttctgccctg tagacactat gtgaataact 3457  
 gaagtaacac tagactgaat ctcttttttg gagtatgtat cttctctcac ttgttcaagt 3517  
 acaggcacac tgttcaaccg catggtatct ttctgttgtg tgacttctac aaatgtaatt 3577  
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 attattttaa attctttcat tttttctgc ctctactat acgactgtag tgcaacaaat 3697  
 attttaaagc ccccttttct tctttatctt cattagtgtg acattgattt cagtgtcaac 3757  
 acatttaaag attcattcat gttgcacagt ggcttcatg aacgtgaaac tgtgatataa 3817  
 ggttttcttt catactcata attagcccaa aacagttgcc aaactttgcc attgtgtccc 3877  
 tgcatttgtg ttgagctgc tatataattg tggaaattac actgaaagt gactaagaga 3937  
 ctattgaaa agcatgaata attaaatata catgtgagag acatctcacc tgctgtattt 3997  
 tacttagtga atattgttca ctctccgtg totgatgtct tgotgaatgc tgtgactcat 4057  
 agtttacttt tgttcaaaat agtttgcact tttgttaat aaaatcaact tgag 4111

<210> 6

<211> 979

<212> PRT

<213> Homo sapiens

<400> 6

Ala	Glu	Pro	Ala	Gly	Arg	Gln	Arg	Pro	Arg	Arg	Glu	Pro	Glu	Ala	Met
1															15

Asp Glu Gln Ser Val Glu Ser Ile Ala Glu Val Phe Arg Cys Phe Ile

20

25

30

Cys Met Glu Lys Leu Arg Asp Ala Arg Leu Cys Pro His Cys Ser Lys  
35 40 45

Leu Cys Cys Phe Ser Cys Ile Arg Arg Trp Leu Thr Glu Gln Arg Ala  
50 55 60

Gln Cys Pro His Cys Arg Ala Pro Leu Gln Leu Arg Glu Leu Val Asn  
65 70 75 80

Cys Arg Trp Ala Glu Glu Val Thr Gln Gln Leu Asp Thr Leu Gln Leu  
85 90 95

Cys Ser Leu Thr Lys His Glu Glu Asn Glu Lys Asp Lys Cys Glu Asn  
100 105 110

His His Glu Lys Leu Ser Val Phe Cys Trp Thr Cys Lys Lys Cys Ile  
115 120 125

Cys His Gln Cys Ala Leu Trp Gly Gly Met His Gly Gly His Thr Phe  
130 135 140

Lys Pro Leu Ala Glu Ile Tyr Glu Gln His Val Thr Lys Val Asn Glu  
145 150 155 160

Glu Val Ala Lys Leu Arg Arg Arg Leu Met Glu Leu Ile Ser Leu Val  
165 170 175

Gln Glu Val Glu Arg Asn Val Glu Ala Val Arg Asn Ala Lys Asp Glu  
180 185 190

Arg Val Arg Glu Ile Arg Asn Ala Val Glu Met Met Ile Ala Arg Leu  
195 200 205

Asp Thr Gln Leu Lys Asn Lys Leu Ile Thr Leu Met Gly Gln Lys Thr  
210 215 220

Ser Leu Thr Gln Glu Thr Glu Leu Leu Glu Ser Leu Leu Gln Glu Val  
225 230 235 240

Glu His Gln Leu Arg Ser Cys Ser Lys Ser Glu Leu Ile Ser Lys Ser  
245 250 255

Ser Glu Ile Leu Met Met Phe Gln Gln Val His Arg Lys Pro Met Ala  
260 265 270

Ser Phe Val Thr Thr Pro Val Pro Pro Asp Phe Thr Ser Glu Leu Val

275	280	285
Pro Ser Tyr Asp Ser Ala Thr Phe Val Leu Glu Asn Phe Ser Thr Leu		
290	295	300
Arg Gln Arg Ala Asp Pro Val Tyr Ser Pro Pro Leu Gln Val Ser Gly		
305	310	315 320
Leu Cys Trp Arg Leu Lys Val Tyr Pro Asp Gly Asn Gly Val Val Arg		
325	330	335
Gly Tyr Tyr Leu Ser Val Phe Leu Glu Leu Ser Ala Gly Leu Pro Glu		
340	345	350
Thr Ser Lys Tyr Glu Tyr Arg Val Glu Met Val His Gln Ser Cys Asn		
355	360	365
Asp Pro Thr Lys Asn Ile Ile Arg Glu Phe Ala Ser Asp Phe Glu Val		
370	375	380
Gly Glu Cys Trp Gly Tyr Asn Arg Phe Phe Arg Leu Asp Leu Leu Ala		
385	390	395 400
Asn Glu Gly Tyr Leu Asn Pro Gln Asn Asp Thr Val Ile Leu Arg Phe		
405	410	415
Gln Val Arg Ser Pro Thr Phe Phe Gln Lys Ser Arg Asp Gln His Trp		
420	425	430
Tyr Ile Thr Gln Leu Glu Ala Ala Gln Thr Ser Tyr Ile Gln Gln Ile		
435	440	445
Asn Asn Leu Lys Glu Arg Leu Thr Ile Glu Leu Ser Arg Thr Gln Lys		
450	455	460
Ser Arg Asp Leu Ser Pro Pro Asp Asn His Leu Ser Pro Gln Asn Asp		
465	470	475 480
Asp Ala Leu Glu Thr Arg Ala Lys Lys Ser Ala Cys Ser Asp Met Leu		
485	490	495
Leu Glu Gly Gly Pro Thr Thr Ala Ser Val Arg Glu Ala Lys Glu Asp		
500	505	510
Glu Glu Asp Glu Glu Lys Ile Gln Asn Glu Asp Tyr His His Glu Leu		
515	520	525
Ser Asp Gly Asp Leu Asp Leu Asp Leu Val Tyr Glu Asp Glu Val Asn		

530

535

540

Gln Leu Asp Gly Ser Ser Ser Ser Ala Ser Ser Thr Ala Thr Ser Asn  
545 550 555 560

Thr Glu Glu Asn Asp Ile Asp Glu Glu Thr Met Ser Gly Glu Asn Asp  
565 570 575

Val Glu Tyr Asn Asn Met Glu Leu Glu Glu Gly Glu Leu Met Glu Asp  
580 585 590

Ala Ala Ala Ala Gly Pro Ala Gly Ser Ser His Gly Tyr Val Gly Ser  
595 600 605

Ser Ser Arg Ile Ser Arg Arg Thr His Leu Cys Ser Ala Ala Thr Ser  
610 615 620

Ser Leu Leu Asp Ile Asp Pro Leu Ile Leu Ile His Leu Leu Asp Leu  
625 630 635 640

Lys Asp Arg Ser Ser Ile Glu Asn Leu Trp Gly Leu Gln Pro Arg Pro  
645 650 655

Pro Ala Ser Leu Leu Gln Pro Thr Ala Ser Tyr Ser Arg Lys Asp Lys  
660 665 670

Asp Gln Arg Lys Gln Gln Ala Met Trp Arg Val Pro Ser Asp Leu Lys  
675 680 685

Met Leu Lys Arg Leu Lys Thr Gln Met Ala Glu Val Arg Cys Met Lys  
690 695 700

Thr Asp Val Lys Asn Thr Leu Ser Glu Ile Lys Ser Ser Ser Ala Ala  
705 710 715 720

Ser Gly Asp Met Gln Thr Ser Leu Phe Ser Ala Asp Gln Ala Ala Leu  
725 730 735

Ala Ala Cys Gly Thr Glu Asn Ser Gly Arg Leu Gln Asp Leu Gly Met  
740 745 750

Glu Leu Leu Ala Lys Ser Ser Val Ala Asn Cys Tyr Ile Arg Asn Ser  
755 760 765

Thr Asn Lys Lys Ser Asn Ser Pro Lys Pro Ala Arg Ser Ser Val Ala  
770 775 780

Gly Ser Leu Ser Leu Arg Arg Ala Val Asp Pro Gly Glu Asn Ser Arg

785		790		795		800
Ser Lys Gly Asp Cys Gln Thr Leu Ser Glu Gly Ser Pro Gly Ser Ser						
	805			810		815
Gln Ser Gly Ser Arg His Ser Ser Pro Arg Ala Leu Ile His Gly Ser						
	820		825			830
Ile Gly Asp Ile Leu Pro Lys Thr Glu Asp Arg Gln Cys Lys Ala Leu						
	835		840			845
Asp Ser Asp Ala Val Val Val Ala Val Phe Ser Gly Leu Pro Ala Val						
	850		855			860
Glu Lys Arg Arg Lys Met Val Thr Leu Gly Ala Asn Ala Lys Gly Gly						
	865		870		875	880
His Leu Glu Gly Leu Gln Met Thr Asp Leu Glu Asn Asn Ser Glu Thr						
	885			890		895
Gly Glu Leu Gln Pro Val Leu Pro Glu Gly Ala Ser Ala Ala Pro Glu						
	900			905		910
Glu Gly Met Ser Ser Asp Ser Asp Ile Glu Cys Asp Thr Glu Asn Glu						
	915			920		925
Glu Gln Glu Glu His Thr Ser Val Gly Gly Phe His Asp Ser Phe Met						
	930		935			940
Val Met Thr Gln Pro Pro Asp Glu Asp Thr His Ser Ser Phe Pro Asp						
	945		950		955	960
Gly Glu Gln Ile Gly Pro Glu Asp Leu Ser Phe Asn Thr Asp Glu Asn						
	965			970		975
Ser Gly Arg						

<210> 7  
 <211> 639  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> CDS  
 <222> (1)..(639)

<400> 7

atg aac cac cag cag cag cag cag cag cag aaa gcg ggc gag cag cag	48
Met Asn His Gln Gln Gln Gln Gln Gln Lys Ala Gly Glu Gln Gln	
1 5 10 15	
ttg agc gag ccc gag gac atg gag atg gaa gcg gga gat aca gat gac	96
Leu Ser Glu Pro Glu Asp Met Glu Met Glu Ala Gly Asp Thr Asp Asp	
20 25 30	
cca cca aga att act cag aac cct gtg atc aat ggg aat gtg gcc ctg	144
Pro Pro Arg Ile Thr Gln Asn Pro Val Ile Asn Gly Asn Val Ala Leu	
35 40 45	
agt gat gga cac aac acc gcg gag gag gac atg gag gat gac acc agt	192
Ser Asp Gly His Asn Thr Ala Glu Glu Asp Met Glu Asp Asp Thr Ser	
50 55 60	
tgg cgc tcc gag gca acc ttt cag ttc act gtg gag cgc ttc agc aga	240
Trp Arg Ser Glu Ala Thr Phe Gln Phe Thr Val Glu Arg Phe Ser Arg	
65 70 75 80	
ctg agt gag tgc gtc ctt agc cct ccg tgt ttt gtg cga aat ctg cca	288
Leu Ser Glu Ser Val Leu Ser Pro Pro Cys Phe Val Arg Asn Leu Pro	
85 90 95	
tgg aag att atg gtg atg cca cgc ttt tat cca gac aga cca cac caa	336
Trp Lys Ile Met Val Met Pro Arg Phe Tyr Pro Asp Arg Pro His Gln	
100 105 110	
aaa agc gta gga ttc ttt ctc cag tgc aat gct gaa tct gat tcc acg	384
Lys Ser Val Gly Phe Phe Leu Gln Cys Asn Ala Glu Ser Asp Ser Thr	
115 120 125	
tca tgg tct tgc cat gca caa gca gtg ctg aag ata ata aat tac aga	432
Ser Trp Ser Cys His Ala Gln Ala Val Leu Lys Ile Ile Asn Tyr Arg	
130 135 140	
gat gat gaa aag tgc ttc agt cgt cgt att agt cat ttg ttc ttc cat	480
Asp Asp Glu Lys Ser Phe Ser Arg Arg Ile Ser His Leu Phe Phe His	
145 150 155 160	
aaa gaa aat gat tgg gga ttt tcc aat ttt atg gcc tgg agt gaa gtg	528
Lys Glu Asn Asp Trp Gly Phe Ser Asn Phe Met Ala Trp Ser Glu Val	
165 170 175	
acc gat cct gag aaa gga ttt ata gat gat gac aaa gtt acc ttt gaa	576
Thr Asp Pro Glu Lys Gly Phe Ile Asp Asp Asp Lys Val Thr Phe Glu	
180 185 190	



gtc ttt gta cag gcg gat gct ccc cat gga gtt gcg tgg gat tca aag 624  
 Val Phe Val Gln Ala Asp Ala Pro His Gly Val Ala Trp Asp Ser Lys  
 195 200 205

aag cac aca ggc tac 639  
 Lys His Thr Gly Tyr  
 210

<210> 8

<211> 213

<212> PRT

<213> Homo sapiens

<400> 8

Met Asn His Gln Gln Gln Gln Gln Lys Ala Gly Glu Gln Gln  
 1 5 10 15

Leu Ser Glu Pro Glu Asp Met Glu Met Glu Ala Gly Asp Thr Asp Asp  
 20 25 30

Pro Pro Arg Ile Thr Gln Asn Pro Val Ile Asn Gly Asn Val Ala Leu  
 35 40 45

Ser Asp Gly His Asn Thr Ala Glu Glu Asp Met Glu Asp Asp Thr Ser  
 50 55 60

Trp Arg Ser Glu Ala Thr Phe Gln Phe Thr Val Glu Arg Phe Ser Arg  
 65 70 75 80

Leu Ser Glu Ser Val Leu Ser Pro Pro Cys Phe Val Arg Asn Leu Pro  
 85 90 95

Trp Lys Ile Met Val Met Pro Arg Phe Tyr Pro Asp Arg Pro His Gln  
 100 105 110

Lys Ser Val Gly Phe Phe Leu Gln Cys Asn Ala Glu Ser Asp Ser Thr  
 115 120 125

Ser Trp Ser Cys His Ala Gln Ala Val Leu Lys Ile Ile Asn Tyr Arg  
 130 135 140

Asp Asp Glu Lys Ser Phe Ser Arg Arg Ile Ser His Leu Phe Phe His  
 145 150 155 160

Lys Glu Asn Asp Trp Gly Phe Ser Asn Phe Met Ala Trp Ser Glu Val  
 165 170 175

Thr Asp Pro Glu Lys Gly Phe Ile Asp Asp Asp Lys Val Thr Phe Glu  
180 185 190

Val Phe Val Gln Ala Asp Ala Pro His Gly Val Ala Trp Asp Ser Lys  
195 200 205

Lys His Thr Gly Tyr  
210

<210> 9

<211> 540

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (1)..(540)

<400> 9

atg tca agg gtt cca agt cct cca cct ccg gca gaa atg tgc agt ggc 48  
Met Ser Arg Val Pro Ser Pro Pro Pro Ala Glu Met Ser Ser Gly  
1 5 10 15

ccc gta gct gag agt tgg tgc tac aca cag atc aag gta tgc aaa ttc 96  
Pro Val Ala Glu Ser Trp Cys Tyr Thr Gln Ile Lys Val Val Lys Phe  
20 25 30

tcc tac atg tgg acc atc aat aac ttt agc ttt tgc cgg gag gaa atg 144  
Ser Tyr Met Trp Thr Ile Asn Asn Phe Ser Phe Cys Arg Glu Glu Met  
35 40 45

ggc gaa gtc att aaa agt tct aca ttt tca tca gga gca aat gat aaa 192  
Gly Glu Val Ile Lys Ser Ser Thr Phe Ser Ser Gly Ala Asn Asp Lys  
50 55 60

ctg aaa tgg tgt ttg cga gta aac ccc aaa ggg tta gat gaa gaa agc 240  
Leu Lys Trp Cys Leu Arg Val Asn Pro Lys Gly Leu Asp Glu Glu Ser  
65 70 75 80

aaa gat tac ctg tca ott tac ctg tta ctg gtc agc tgt cca aag agt 288  
Lys Asp Tyr Leu Ser Leu Tyr Leu Leu Val Ser Cys Pro Lys Ser  
85 90 95

gaa gtt ccg gca aaa ttc aaa ttc tcc atc ctg aat gcc aag gga gaa 336  
Glu Val Arg Ala Lys Phe Lys Phe Ser Ile Leu Asn Ala Lys Gly Glu  
100 105 110

gaa acc aaa gct atg gag agt caa cgg gca tat agg ttt gtg caa ggc 384  
 Glu Thr Lys Ala Met Glu Ser Gln Arg Ala Tyr Arg Phe Val Gln Gly  
 115 120 125

aaa gac tgg gga ttc aag aaa ttc atc cgt aga gat ttt ctt ttg gat 432  
 Lys Asp Trp Gly Phe Lys Lys Phe Ile Arg Arg Asp Phe Leu Leu Asp  
 130 135 140

gag gcc aac ggg ctt ctc cct gat gac aag ctt acc ctc ttc tgc gag 480  
 Glu Ala Asn Gly Leu Leu Pro Asp Asp Lys Leu Thr Leu Phe Cys Glu  
 145 150 155 160

gtg agt gtt gtg caa gat tct gtc aac att tct ggc cag aat acc atg 528  
 Val Ser Val Val Gln Asp Ser Val Asn Ile Ser Gly Gln Asn Thr Met  
 165 170 175

aac atg gta aag 540  
 Asn Met Val Lys  
 180

<210> 10

<211> 180

<212> PRT

<213> Homo sapiens

<400> 10

Met Ser Arg Val Pro Ser Pro Pro Pro Pro Ala Glu Met Ser Ser Gly  
 1 5 10 15

Pro Val Ala Glu Ser Trp Cys Tyr Thr Gln Ile Lys Val Val Lys Phe  
 20 25 30

Ser Tyr Met Trp Thr Ile Asn Asn Phe Ser Phe Cys Arg Glu Glu Met  
 35 40 45

Gly Glu Val Ile Lys Ser Ser Thr Phe Ser Ser Gly Ala Asn Asp Lys  
 50 55 60

Leu Lys Trp Cys Leu Arg Val Asn Pro Lys Gly Leu Asp Glu Glu Ser  
 65 70 75 80

Lys Asp Tyr Leu Ser Leu Tyr Leu Leu Leu Val Ser Cys Pro Lys Ser  
 85 90 95

Glu Val Arg Ala Lys Phe Lys Phe Ser Ile Leu Asn Ala Lys Gly Glu  
 100 105 110

Glu Thr Lys Ala Met Glu Ser Gln Arg Ala Tyr Arg Phe Val Gln Gly  
 115 120 125  
 Lys Asp Trp Gly Phe Lys Lys Phe Ile Arg Arg Asp Phe Leu Leu Asp  
 130 135 140  
 Glu Ala Asn Gly Leu Leu Pro Asp Asp Lys Leu Thr Leu Phe Cys Glu  
 145 150 155 160  
 Val Ser Val Val Gln Asp Ser Val Asn Ile Ser Gly Gln Asn Thr Met  
 165 170 175  
 Asn Met Val Lys  
 180

<210> 11  
 <211> 459  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> CDS  
 <222> (1)..(459)

<400> 11  
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 Phe Thr Ser Glu Leu Val Pro Ser Tyr Asp Ser Ala Thr Phe Val Leu  
 1 5 10 15  
 gag aat ttc agc act ttg cgt cag aga gca gat cct gtt tac agt cca 96  
 Glu Asn Phe Ser Thr Leu Arg Gln Arg Ala Asp Pro Val Tyr Ser Pro  
 20 25 30  
 cct ctt caa gtt tca gga ctt tgc tgg agg tta aaa gtt tac cca gat 144  
 Pro Leu Gln Val Ser Gly Leu Cys Trp Arg Leu Lys Val Tyr Pro Asp  
 35 40 45  
 gga aat gga gtt gtg cga ggt tac tac tta tct gtg ttt ctg gag ctc 192  
 Gly Asn Gly Val Val Arg Gly Tyr Leu Ser Val Phe Leu Glu Leu  
 50 55 60  
 tca gct ggc ttg cct gaa act tct aaa tat gaa tat cgt gta gag atg 240  
 Ser Ala Gly Leu Pro Glu Thr Ser Lys Tyr Glu Tyr Arg Val Glu Met  
 65 70 75 80  
 gtt cac cag tcc tgt aat gat cct aca aaa aat atc att cga gaa ttt 288

Val His Gln Ser Cys Asn Asp Pro Thr Lys Asn Ile Ile Arg Glu Phe  
                     85                    90                    95  
  
 gca tct gac ttt gaa gtt gga gaa tgc tgg ggc tat aat aga ttt ttc 336  
 Ala Ser Asp Phe Glu Val Gly Glu Cys Trp Gly Tyr Asn Arg Phe Phe  
                     100                    105                    110  
  
 cgt ttg gac tta ctc gca aat gaa gga tac ttg aat cca caa aat gat 384  
 Arg Leu Asp Leu Leu Ala Asn Glu Gly Tyr Leu Asn Pro Gln Asn Asp  
                     115                    120                    125  
  
 aca gtg att tta agg ttt cag gta cgt tca cca act ttc ttt caa aaa 432  
 Thr Val Ile Leu Arg Phe Gln Val Arg Ser Pro Thr Phe Phe Gln Lys  
                     130                    135                    140  
  
 tcc cgg gac cag cat tgg tac att act  
 Ser Arg Asp Gln His Trp Tyr Ile Thr 459  
 145                    150

<210> 12  
 <211> 153  
 <212> PRT  
 <213> Homo sapiens

<400> 12  
 Phe Thr Ser Glu Leu Val Pro Ser Tyr Asp Ser Ala Thr Phe Val Leu  
   1                    5                    10                    15  
  
 Glu Asn Phe Ser Thr Leu Arg Gln Arg Ala Asp Pro Val Tyr Ser Pro  
                     20                    25                    30  
  
 Pro Leu Gln Val Ser Gly Leu Cys Trp Arg Leu Lys Val Tyr Pro Asp  
                     35                    40                    45  
  
 Gly Asn Gly Val Val Arg Gly Tyr Tyr Leu Ser Val Phe Leu Glu Leu  
   50                    55                    60  
  
 Ser Ala Gly Leu Pro Glu Thr Ser Lys Tyr Glu Tyr Arg Val Glu Met  
   65                    70                    75                    80  
  
 Val His Gln Ser Cys Asn Asp Pro Thr Lys Asn Ile Ile Arg Glu Phe  
                     85                    90                    95  
  
 Ala Ser Asp Phe Glu Val Gly Glu Cys Trp Gly Tyr Asn Arg Phe Phe  
                     100                    105                    110  
  
 Arg Leu Asp Leu Leu Ala Asn Glu Gly Tyr Leu Asn Pro Gln Asn Asp

115

120

125

Thr Val Ile Leu Arg Phe Gln Val Arg Ser Pro Thr Phe Phe Gln Lys  
 130 135 140

Ser Arg Asp Gln His Trp Tyr Ile Thr  
 145 150

&lt;210&gt; 13

&lt;211&gt; 17

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: Primer

&lt;400&gt; 13

gcgaattcca ggccgcg

17

&lt;210&gt; 14

&lt;211&gt; 27

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: Primer

&lt;400&gt; 14

ttcctcgagc cgacttagcc tgtgtgc

27

&lt;210&gt; 15

&lt;211&gt; 27

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: Primer

&lt;400&gt; 15

cttcgaattc gcgatgtcaa gggttcc

27

&lt;210&gt; 16

&lt;211&gt; 29

&lt;212&gt; DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 16

ccatgctcga ggtattctag ccagaaatg

29

<210> 17

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 17

ccagaattca ccagtgaatt agtgcc

26

<210> 18

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 18

ccaactcgagt aatgtaccaa tgctagtcc

29

<210> 19

<211> 94

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Consensus  
Sequence

<220>

<221> SITE

<222> (2)..(22)

<223> Xaa = any amino acid

<220>

<221> UNSURE  
<222> (19)..(22)  
<223> Xaa may or may not be present

<220>  
<221> SITE  
<222> (24)  
<223> Xaa=any amino acid

<220>  
<221> SITE  
<222> (26)..(28)  
<223> Xaa=any amino acid

<220>  
<221> SITE  
<222> (30)  
<223> Xaa=any amino acid

<220>  
<221> SITE  
<222> (32)..(47)  
<223> Xaa=any amino acid

<220>  
<221> UNSURE  
<222> (47)  
<223> Xaa may or may not be present

<220>  
<221> SITE  
<222> (49)..(76)  
<223> Xaa=any amino acid

<220>  
<221> UNSURE  
<222> (73)..(76)  
<223> Xaa may or may not be present

<220>  
<221> SITE  
<222> (78)..(93)  
<223> Xaa=any amino acid

<220>  
<221> UNSURE  
<222> (93)  
<223> Xaa may or may not be present



<400> 19

Glu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Trp Xaa Xaa Xaa Val Xaa Pro Xaa  
20 25 30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu  
35 40 45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
50 55 60

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Lys Xaa Xaa Xaa  
65 70 75 80

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Trp  
85 90

<210> 20

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<221> SITE

<222> (2)

<223> Xaa =any amino acid

<220>

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<222> (4)

<223> Xaa=any amino acid

<220>

<221> SITE

<222> (5)

<223> Xaa =Leu or Ile

<220>

<221> SITE

<222> (6)

<223> Xaa =any amino acid

<220>

<221> SITE

<222> {8}  
<223> Xaa = any amino acid

<220>  
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Sequence

<400> 20  
Leu Xaa Trp Xaa Xaa Xaa Val Xaa Pro  
1 5

<210> 21  
<211> 124  
<212> PRT  
<213> Artificial Sequence

<220>  
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<222> (12)..(14)  
<223> Xaa may or may not be present

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<222> (16)..(22)  
<223> Xaa=any amino acid

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<222> (26)..(28)  
<223> Xaa=any amino acid

<220>  
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<222> (32)..(42)  
<223> Xaa = any amino acid

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<222> (42)  
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<222> (73)..(76)
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<222> (78)..(87)
<223> Xaa = any amino acid

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<222> (87)
<223> Xaa may or may not be present

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<222> (89)..(93)
<223> Xaa=any amino acid

<220>
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<222> (96)..(98)
<223> Xaa=any amino acid

<220>
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<222> (100)..(115)
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<220>
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<222> (117)..(123)
<223> Xaa=any amino acid

<220>
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<222> (122)..(123)
<223> Xaa may or may not be present

<220>
<223> Description of Artificial Sequence: Consensus
Sequence

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 <223> Xaa may or may not be present

<220>  
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 <222> (24)  
 <223> Xaa=any amino acid

<220>  
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 <223> Xaa=any amino acid

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 <223> Xaa = any amino acid

<400> 21  
 Glu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ser Xaa  
 1 5 10 15  
 Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Trp Xaa Xaa Xaa Val Xaa Pro Xaa  
 20 25 30  
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ser Xaa Xaa Xaa Xaa Leu  
 35 40 45  
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
 50 55 60  
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Lys Xaa Xaa Xaa  
 65 70 75 80  
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Phe Xaa Xaa Xaa Xaa Xaa Trp Gly Xaa  
 85 90 95  
 Xaa Xaa Phe Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
 100 105 110  
 Xaa Xaa Xaa Asp Xaa Xaa Xaa Xaa Xaa Xaa Xaa Val  
 115 120

<210> 22  
 <211> 124

<212> PRT  
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<220>  
<221> SITE  
<222> (6)  
<223> Xaa = Val, Leu, or Ile

<220>  
<221> SITE  
<222> (7)..(14)  
<223> Xaa =any amino acid

<220>  
<221> SITE  
<222> (16)  
<223> Xaa=any amino acid

<220>  
<221> SITE  
<222> (17)  
<223> Xaa = Pro or Gly

<220>  
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<222> (18)..(22)  
<223> Xaa =any amino acid

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<223> Xaa may or may not be present

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<222> (24)  
<223> Xaa =any amino acid

<220>  
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<222> (26)  
<223> Xaa =any amino acid

<220>  
<221> SITE  
<222> (27)  
<223> Xaa =Val, Leu or Ile

<220>  
<221> SITE  
<222> (28)  
<223> Xaa = any amino acid

<220>  
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<223> Xaa = any amino acid

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<221> UNSURE  
<222> (42)..(42)  
<223> Xaa may or may not be present

<220>  
<221> SITE  
<222> (44)  
<223> Xaa =Val, Leu or Ile

<220>  
<221> SITE  
<222> (45)..(47)  
<223> Xaa=any amino acid

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<222> (49)..(66)  
<223> Xaa=any amino acid

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<223> Xaa=Val, Leu or Ile

<220>

<221> SITE

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<223> Xaa = any amino acid

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<221> SITE

<222> (74)

<223> Xaa=Asp, Glu, Asn or Gln

<220>

<221> SITE

<222> (75)..(76)

<223> Xaa = any amino acid

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<223> Description of Artificial Sequence: Consensus  
Sequence

<220>

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<223> Xaa may or may not be present

<220>

<221> UNSURE

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<223> Xaa may or may not be present

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<221> SITE

<222> (78)..(87)

<223> Xaa = any amino acid

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<221> UNSURE

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<223> Xaa may or may not be present

<220>

<221> SITE

<222> (89)..(93)

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<221> SITE

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<222> (96)
<223> Xaa=Trp or Phe

<220>
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<222> (97)..(98)
<223> Xaa=any amino acid

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<223> Xaa = any amino acid

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<222> (105)
<223> Xaa=Val, Leu or Ile

<220>
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<222> (106)
<223> Xaa=any amino acid

<220>
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<222> (107)
<223> Xaa=Asp, Glu, Asn or Gln

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<222> (108)..(114)
<223> Xaa=any amino acid

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<222> (115)
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<222> (117)
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<220>
<221> SITE
<222> (118)
<223> Xaa=Val, Leu or Ile

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<220>  
 <221> SITE  
 <222> (119)..(122)  
 <223> Xaa=any amino acid

<220>  
 <221> UNSURE  
 <222> (121)..(122)  
 <223> Xaa may or may not be present

<220>  
 <221> SITE  
 <222> (123)  
 <223> Xaa=Asp, Glu, Asn or Gln

<400> 22  
 Glu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ser Xaa  
 1 5 10 15  
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
 20 25 30  
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
 35 40 45  
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
 50 55 60  
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
 65 70 75 80  
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
 85 90 95  
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
 100 105 110  
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
 115 120

<210> 23  
 <211> 139  
 <212> PRT  
 <213> Homo sapiens

<400> 23  
 Asp Met Glu Asp Asp Thr Ser Trp Arg Ser Glu Ala Thr Phe Gln Phe

1	5	10	15
Thr Val Glu Arg Phe Ser Arg Leu Ser Glu Ser Val Leu Ser Pro Pro	20	25	30
Cys Phe Val Arg Asn Leu Pro Trp Lys Ile Met Val Met Pro Arg Phe	35	40	45
Tyr Pro Asp Arg Pro His Gln Lys Ser Val Gly Phe Phe Leu Gln Cys	50	55	60
Asn Ala Glu Ser Asp Ser Thr Ser Trp Ser Cys His Ala Gln Ala Val	65	70	75
Leu Lys Ile Ile Asn Tyr Arg Asp Asp Glu Lys Ser Phe Ser Arg Arg	85	90	95
Ile Ser His Leu Phe Phe His Lys Glu Asn Asp Trp Gly Phe Ser Asn	100	105	110
Phe Met Ala Trp Ser Glu Val Thr Asp Pro Glu Lys Gly Phe Ile Asp	115	120	125
Asp Asp Lys Val Thr Phe Glu Val Phe Val Gln	130	135	
<210> 24			
<211> 132			
<212> PRT			
<213> Homo sapiens			
<400> 24			
Ser Tyr Met Trp Thr Ile Asn Asn Phe Ser Phe Cys Arg Glu Glu Met	1	5	10
Gly Glu Val Ile Lys Ser Ser Thr Phe Ser Ser Gly Ala Asn Asp Lys	20	25	30
Leu Lys Trp Cys Leu Arg Val Asn Pro Lys Gly Leu Asp Glu Glu Ser	35	40	45
Lys Asp Tyr Leu Ser Leu Tyr Leu Leu Leu Val Ser Cys Pro Lys Ser	50	55	60
Glu Val Arg Ala Lys Phe Lys Phe Ser Ile Leu Asn Ala Lys Gly Glu	65	70	75
			80

Glu Thr Lys Ala Met Glu Ser Gln Arg Ala Tyr Arg Phe Val Gln Gly  
85 90 95

Lys Asp Trp Gly Phe Lys Lys Phe Ile Arg Arg Asp Phe Leu Leu Asp  
100 105 110

Glu Ala Asn Gly Leu Leu Pro Asp Asp Lys Leu Thr Leu Phe Cys Glu  
115 120 125

Val Ser Val Val  
130

<210> 25

<211> 135

<212> PRT

<213> Homo sapiens

<400> 25

Glu Leu Val Pro Ser Tyr Asp Ser Ala Thr Phe Val Leu Glu Asn Phe  
1 5 10 15

Ser Thr Leu Arg Gln Arg Ala Asp Pro Val Tyr Ser Pro Pro Leu Gln  
20 25 30

Val Ser Gly Leu Cys Trp Arg Leu Lys Val Tyr Pro Asp Gly Asn Gly  
35 40 45

Val Val Arg Gly Tyr Tyr Leu Ser Val Phe Leu Glu Leu Ser Ala Gly  
50 55 60

Leu Pro Glu Thr Ser Lys Tyr Glu Tyr Arg Val Glu Met Val His Gln  
65 70 75 80

Ser Cys Asn Asp Pro Thr Lys Asn Ile Ile Arg Glu Phe Ala Ser Asp  
85 90 95

Phe Glu Val Gly Glu Cys Trp Gly Tyr Asn Arg Phe Phe Arg Leu Asp  
100 105 110

Leu Leu Ala Asn Glu Gly Tyr Leu Asn Pro Gln Asn Asp Thr Val Ile  
115 120 125

Leu Arg Phe Gln Val Arg Ser  
130 135

<210> 26

<211> 150  
 <212> PRT  
 <213> Homo sapiens

<400> 26

Gly Thr Phe Leu Trp Lys Ile Thr Asn Val Thr Arg Arg Cys His Glu  
 1 5 10 15

Ser Ala Cys Gly Arg Thr Val Ser Leu Phe Ser Pro Ala Phe Tyr Thr  
 20 25 30

Ala Lys Tyr Gly Tyr Lys Leu Cys Leu Arg Leu Tyr Leu Asn Gly Asp  
 35 40 45

Gly Thr Gly Lys Arg Thr His Leu Ser Leu Phe Ile Val Ile Met Arg  
 50 55 60

Gly Glu Tyr Asp Ala Leu Leu Pro Trp Pro Phe Arg Asn Lys Val Thr  
 65 70 75 80

Phe Met Leu Leu Asp Gln Asn Asn Arg Glu His Ala Ile Asp Ala Phe  
 85 90 95

Arg Pro Asp Leu Ser Ser Ala Ser Phe Gln Arg Pro Gln Ser Glu Thr  
 100 105 110

Asn Val Ala Ser Gly Cys Pro Leu Phe Phe Pro Leu Ser Lys Leu Gln  
 115 120 125

Ser Pro Lys His Ala Tyr Val Lys Asp Asp Thr Met Phe Leu Lys Cys  
 130 135 140

Ile Val Glu Thr Ser Thr  
 145 150

<210> 27  
 <211> 150  
 <212> PRT  
 <213> Homo sapiens

<400> 27

Gly Val Phe Ile Trp Lys Ile Ser Asp Phe Ala Arg Lys Arg Gln Glu  
 1 5 10 15

Ala Val Ala Gly Arg Ile Pro Ala Ile Phe Ser Pro Ala Phe Tyr Thr  
 20 25 30

Ser Arg Tyr Gly Tyr Lys Met Cys Leu Arg Ile Tyr Leu Asn Gly Asp  
35 40 45

Gly Thr Gly Arg Gly Thr His Leu Ser Leu Phe Phe Val Val Met Lys  
50 55 60

Gly Pro Asn Asp Ala Leu Leu Arg Trp Pro Phe Asn Gln Lys Val Thr  
65 70 75 80

Leu Met Leu Leu Asp Gln Asn Asn Arg Glu His Val Ile Asp Ala Phe  
85 90 95

Arg Pro Asp Val Thr Ser Ser Ser Phe Gln Arg Pro Val Asn Asp Met  
100 105 110

Asn Ile Ala Ser Gly Cys Pro Leu Phe Cys Pro Val Ser Lys Met Glu  
115 120 125

Ala Lys Asn Ser Tyr Val Arg Asp Asp Ala Ile Phe Ile Lys Ala Ile  
130 135 140

Val Asp Leu Thr Gly Leu  
145 150

<210> 28

<211> 153

<212> PRT

<213> Homo sapiens

<400> 28

Gly Val Leu Ile Trp Lys Ile Arg Asp Tyr Lys Arg Arg Lys Gln Glu  
1 5 10 15

Ala Val Met Gly Lys Thr Leu Ser Leu Tyr Ser Gln Pro Phe Tyr Thr  
20 25 30

Gly Tyr Phe Gly Tyr Lys Met Cys Ala Arg Val Tyr Leu Asn Gly Asp  
35 40 45

Gly Met Gly Lys Gly Thr His Leu Ser Leu Phe Phe Val Ile Met Arg  
50 55 60

Gly Glu Tyr Asp Ala Leu Leu Pro Trp Pro Phe Lys Gln Lys Val Thr  
65 70 75 80

Leu Met Leu Met Asp Gln Gly Ser Ser Arg Arg His Leu Gly Asp Ala  
85 90 95

Phe Lys Pro Asp Pro Asn Ser Ser Ser Phe Lys Lys Pro Ile Gly Glu  
100 105 110

Met Asn Ile Ala Ser Gly Cys Pro Val Phe Val Ala Gln Thr Val Leu  
115 120 125

Glu Asn Gly Thr Tyr Ile Lys Asp Asp Thr Ile Phe Ile Lys Val Ile  
130 135 140

Val Asp Thr Ser Asp Leu Pro Asp Pro  
145 150

<210> 29

<211> 163

<212> PRT

<213> Homo sapiens

<400> 29

Gly Val Leu Ile Trp Lys Ile Gly Ser Tyr Gly Arg Arg Leu Gln Glu  
1 5 10 15

Ala Lys Ala Lys Pro Asn Leu Glu Cys Phe Ser Pro Ala Phe Tyr Thr  
20 25 30

His Lys Tyr Gly Tyr Lys Leu Gln Val Ser Ala Phe Leu Asn Gly Asn  
35 40 45

Gly Ser Gly Glu Gly Thr His Leu Ser Leu Tyr Ile Arg Val Leu Pro  
50 55 60

Gly Ala Phe Asp Asn Leu Leu Glu Trp Pro Phe Ala Arg Arg Val Thr  
65 70 75 80

Phe Ser Leu Leu Asp Gln Ser Asp Pro Gly Leu Ala Lys Pro Gln His  
85 90 95

Val Thr Glu Thr Phe His Pro Asp Pro Asn Trp Lys Asn Phe Gln Lys  
100 105 110

Pro Gly Thr Trp Arg Gly Ser Leu Asp Glu Ser Ser Leu Gly Phe Gly  
115 120 125

Tyr Pro Lys Phe Ile Ser His Gln Asp Ile Arg Lys Arg Asn Tyr Val  
130 135 140

Arg Asp Asp Ala Val Phe Ile Arg Ala Ala Val Glu Leu Pro Arg Lys

145

150

155

160

Ile Leu Ser

&lt;210&gt; 30

&lt;211&gt; 154

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 30

Gly Lys Leu Ile Trp Lys Val Thr Asp Tyr Lys Met Lys Lys Arg Glu  
 1 5 10 15

Ala Val Asp Gly His Thr Val Ser Ile Phe Ser Gln Ser Phe Tyr Thr  
 20 25 30

Ser Arg Cys Gly Tyr Arg Leu Cys Ala Arg Ala Tyr Leu Asn Gly Asp  
 35 40 45

Gly Ser Gly Arg Gly Ser His Leu Ser Leu Tyr Phe Val Val Met Arg  
 50 55 60

Gly Glu Phe Asp Ser Leu Leu Gln Trp Pro Phe Arg Gln Arg Val Thr  
 65 70 75 80

Leu Met Leu Leu Asp Gln Ser Gly Lys Lys Asn Ile Met Glu Thr Phe  
 85 90 95

Lys Pro Asp Pro Asn Ser Ser Ser Phe Lys Arg Pro Asp Gly Glu Met  
 100 105 110

Asn Ile Ala Ser Gly Cys Pro Arg Phe Val Ala His Ser Val Leu Glu  
 115 120 125

Asn Ala Lys Asn Ala Tyr Ile Lys Asp Asp Thr Leu Phe Leu Lys Val  
 130 135 140

Ala Val Asp Leu Thr Asp Leu Glu Asp Leu  
 145 150

&lt;210&gt; 31

&lt;211&gt; 157

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

<400> 31

Gly Ile Tyr Ile Trp Lys Ile Gly Asn Phe Gly Met His Leu Lys Cys  
1 5 10 15

Gln Glu Glu Glu Lys Pro Val Val Ile His Ser Pro Gly Phe Tyr Thr  
20 25 30

Gly Lys Pro Gly Tyr Lys Leu Cys Met Arg Leu His Leu Gln Leu Pro  
35 40 45

Thr Ala Gln Arg Cys Ala Asn Tyr Ile Ser Leu Phe Val His Thr Met  
50 55 60

Gln Gly Glu Tyr Asp Ser His Leu Pro Trp Pro Phe Gln Gly Thr Ile  
65 70 75 80

Arg Leu Thr Ile Leu Asp Gln Ser Glu Ala Pro Val Arg Gln Asn His  
85 90 95

Glu Glu Ile Met Asp Ala Lys Pro Glu Leu Leu Ala Phe Gln Arg Pro  
100 105 110

Thr Ile Pro Arg Asn Pro Lys Gly Phe Gly Tyr Val Thr Phe Met His  
115 120 125

Leu Glu Ala Leu Arg Gln Arg Thr Phe Ile Lys Asp Asp Thr Leu Leu  
130 135 140

Val Arg Cys Glu Val Ser Thr Arg Phe Asp Met Gly Ser  
145 150 155

<210> 32

<211> 964

<212> PRT

<213> Homo sapiens

<400> 32

Met Asp Glu Gln Ser Val Glu Ser Ile Ala Glu Val Phe Arg Cys Phe  
1 5 10 15

Ile Cys Met Glu Lys Leu Arg Asp Ala Arg Leu Cys Pro His Cys Ser  
20 25 30

Lys Leu Cys Cys Phe Ser Cys Ile Arg Arg Trp Leu Thr Glu Gln Arg  
35 40 45

Ala Gln Cys Pro His Cys Arg Ala Pro Leu Gln Leu Arg Glu Leu Val



50

55

60

Asn Cys Arg Trp Ala Glu Glu Val Thr Gln Gln Leu Asp Thr Leu Gln  
65 70 75 80

Leu Cys Ser Leu Thr Lys His Glu Glu Asn Glu Lys Asp Lys Cys Glu  
85 90 95

Asn His His Glu Lys Leu Ser Val Phe Cys Trp Thr Cys Lys Lys Cys  
100 105 110

Ile Cys His Gln Cys Ala Leu Trp Gly Gly Met His Gly Gly His Thr  
115 120 125

Phe Lys Pro Leu Ala Glu Ile Tyr Glu Gln His Val Thr Lys Val Asn  
130 135 140

Glu Glu Val Ala Lys Leu Arg Arg Arg Leu Met Glu Leu Ile Ser Leu  
145 150 155 160

Val Gln Glu Val Glu Arg Asn Val Glu Ala Val Arg Asn Ala Lys Asp  
165 170 175

Glu Arg Val Arg Glu Ile Arg Asn Ala Val Glu Met Met Ile Ala Arg  
180 185 190

Leu Asp Thr Gln Leu Lys Asn Lys Leu Ile Thr Leu Met Gly Gln Lys  
195 200 205

Thr Ser Leu Thr Gln Glu Thr Glu Leu Leu Glu Ser Leu Leu Gln Glu  
210 215 220

Val Glu His Gln Leu Arg Ser Cys Ser Lys Ser Glu Leu Ile Ser Lys  
225 230 235 240

Ser Ser Glu Ile Leu Met Met Phe Gln Gln Val His Arg Lys Pro Met  
245 250 255

Ala Ser Phe Val Thr Thr Pro Val Pro Pro Asp Phe Thr Ser Glu Leu  
260 265 270

Val Pro Ser Tyr Asp Ser Ala Thr Phe Val Leu Glu Asn Phe Ser Thr  
275 280 285

Leu Arg Gln Arg Ala Asp Pro Val Tyr Ser Pro Pro Leu Gln Val Ser  
290 295 300

Gly Leu Cys Trp Arg Leu Lys Val Tyr Pro Asp Gly Asn Gly Val Val

305		310		315		320
Arg Gly Tyr Tyr Leu Ser Val Phe Leu Glu Leu Ser Ala Gly Leu Pro						
	325		330			335
Glu Thr Ser Lys Tyr Glu Tyr Arg Val Glu Met Val His Gln Ser Cys						
	340		345			350
Asn Asp Pro Thr Lys Asn Ile Ile Arg Cys Phe Ala Ser Asp Phe Glu						
	355		360			365
Val Gly Glu Cys Trp Gly Tyr Asn Arg Phe Phe Arg Leu Asp Leu Leu						
	370		375			380
Ala Asn Glu Gly Tyr Leu Asn Pro Gln Asn Asp Thr Val Ile Leu Arg						
	385		390			395
						400
Phe Gln Val Arg Ser Pro Thr Phe Phe Gln Lys Ser Arg Asp Gln His						
	405				410	415
Trp Tyr Thr Ile Gln Leu Glu Ala Ala Gln Thr Ser Tyr Ile Gln Gln						
	420				425	430
Ile Asn Asn Leu Lys Glu Arg Leu Thr Ile Glu Leu Ser Arg Thr Gln						
	435				440	445
Lys Ser Arg Asp Leu Ser Pro Pro Asp Asn His Leu Ser Pro Gln Asn						
	450				455	460
Asp Asp Ala Leu Glu Thr Arg Ala Lys Lys Ser Ala Cys Ser Asp Met						
	465		470		475	480
Leu Leu Glu Gly Gly Pro Thr Thr Ala Ser Val Arg Glu Ala Lys Glu						
	485				490	495
Asp Glu Glu Asp Glu Glu Lys Ile Gln Asn Glu Asp Tyr His His Glu						
	500				505	510
Leu Ser Asp Gly Asp Leu Asp Leu Asp Leu Val Tyr Glu Asp Glu Val						
	515				520	525
Asn Gln Leu Asp Gly Ser Ser Ser Ser Ala Ser Ser Thr Ala Thr Ser						
	530				535	540
Asn Thr Glu Glu Asn Asp Ile Asp Glu Glu Thr Met Ser Gly Glu Asn						
	545				550	555
						560
Asp Val Glu Tyr Asn Asn Met Glu Leu Glu Glu Gly Glu Leu Met Glu						

	565		570		575										
Asp	Ala	Ala	Ala	Gly	Pro	Ala	Gly	Ser	Ser	His	Gly	Tyr	Val	Gly	
	580						585					590			
Ser	Ser	Ser	Arg	Ile	Ser	Arg	Arg	Thr	His	Leu	Cys	Ser	Ala	Ala	Thr
	595						600					605			
Ser	Ser	Leu	Leu	Asp	Ile	Asp	Pro	Leu	Ile	Leu	Ile	His	Leu	Leu	Asp
	610						615					620			
Leu	Lys	Asp	Arg	Ser	Ser	Ile	Glu	Asn	Leu	Trp	Gly	Leu	Gln	Pro	Arg
625						630					635			640	
Pro	Pro	Ala	Ser	Leu	Leu	Gln	Pro	Thr	Ala	Ser	Tyr	Ser	Arg	Lys	Asp
						645					650			655	
Lys	Asp	Gln	Arg	Lys	Gln	Gln	Ala	Met	Trp	Arg	Val	Pro	Ser	Asp	Leu
						660								670	
Lys	Met	Leu	Lys	Arg	Leu	Lys	Thr	Gln	Met	Ala	Glu	Val	Arg	Cys	Met
		675					680							685	
Lys	Thr	Asp	Val	Lys	Asn	Thr	Leu	Ser	Glu	Ile	Lys	Ser	Ser	Ser	Ala
		690					695							700	
Ala	Ser	Gly	Asp	Met	Gln	Thr	Ser	Leu	Phe	Ser	Ala	Asp	Gln	Ala	Ala
705						710					715				720
Leu	Ala	Ala	Cys	Gly	Thr	Glu	Asn	Ser	Gly	Arg	Leu	Gln	Asp	Leu	Gly
						725					730			735	
Met	Glu	Leu	Leu	Ala	Lys	Ser	Ser	Val	Ala	Asn	Cys	Tyr	Ile	Arg	Asn
						740					745			750	
Ser	Thr	Asn	Lys	Lys	Ser	Asn	Ser	Pro	Lys	Pro	Ala	Arg	Ser	Ser	Val
			755											760	
Ala	Gly	Ser	Leu	Ser	Leu	Arg	Arg	Ala	Val	Asp	Pro	Gly	Glu	Asn	Ser
			770											775	
Arg	Ser	Lys	Gly	Asp	Cys	Gln	Thr	Leu	Ser	Glu	Gly	Ser	Pro	Gly	Ser
785						790					795				800
Ser	Gln	Ser	Gly	Ser	Arg	His	Ser	Ser	Pro	Arg	Ala	Leu	Ile	His	Gly
						805								810	
Ser	Ile	Gly	Asp	Ile	Leu	Pro	Lys	Thr	Glu	Asp	Arg	Gln	Cys	Lys	Ala

820

825

830

Leu Asp Ser Asp Ala Val Val Val Ala Val Phe Ser Gly Leu Pro Ala  
 835 840 845

Val Glu Lys Arg Arg Lys Met Val Thr Leu Gly Ala Asn Ala Lys Gly  
 850 855 860

Gly His Leu Phe Gly Leu Gln Met Thr Asp Leu Glu Asn Asn Ser Glu  
 865 870 875 880

Thr Gly Phe Leu Gln Pro Val Leu Pro Glu Gly Ala Ser Ala Ala Pro  
 885 890 895

Glu Glu Gly Met Ser Ser Asp Ser Asp Ile Glu Gly Asp Thr Glu Asn  
 900 905 910

Glu Glu Gln Glu Glu His Thr Ser Val Gly Gly Phe His Asp Ser Phe  
 915 920 925

Met Val Met Thr Gln Pro Pro Asp Glu Asp Thr His Ser Ser Glu Pro  
 930 935 940

Asp Gly Phe Gln Ile Gly Pro Glu Asp Leu Ser Phe Asn Thr Asp Glu  
 945 950 955 960

Asn Ser Gly Arg